INHIBITING EFFECT OF ACETIC ACID UPON MICRO-ORGANISMS IN THE PRESENCE OF SODIUM CHLORIDE AND SUCROSE[1,2]

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Vinegar has long been added to foods in the home and in the commercial packing plant as a flavoring element; in addition, it has a definite preservative action against spoilage microorganisms. Any toxic effect of vinegar is undoubtedly due to its acetic acid content.

Levine and Fellers (1940) showed that the toxicity of acetic acid is not due to pH alone although an increase in the hydrogen ions resulted in a decrease in heat resistance of the bacteria studied.

As vinegar is usually associated with sugar or salt or both in a food, it seemed advisable to study the toxic effect of acetic acid in the presence of these substances.

LITERATURE REVIEW

The uses of vinegar in foods are legion: mayonnaise dressings, prepared mustard, horse-radish, pickles, salads, marinated fish, spinach, and beets are only a few of the products with which it is used. Rowse (1928) attributed the keeping qualities of mayonnaise to its vinegar content and pointed out that the preserving power of vinegar varies directly with its acetic acid content. Pederson and Breed (1926) found that one per cent acetic acid

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stopped the growth in tomato catsup of all microorganisms studied and that combinations of salt and sugar with the acid did not lower appreciably the amount of acetic acid required.

The antiseptic action of acetic acid and some other organic acids is not confined to bacteria; these acids appear to be toxic to yeasts and molds as well. Cruess and Hascal (1924) reported that 0.8 to 1.0 per cent acetic acid seemed to inhibit the fermentation of apple cider by Burgundy yeast. Katagiri (1926) found that at a constant concentration of free acid, the rate of fermentation by yeast was independent of the concentration of either acetate or formate salts. Similarly, the nature of the salt cation, Na, K, or NH_4 had no influence. The yeast fermentation was, however, very sensitive to the presence of free acid. This investigator states that the effect is no doubt a complex one, due in part to hydrogen ions and in part to the undissociated molecules of the acid but that the influence of the latter is much greater than that of the former.

Fabian and Wadsworth (1939) demonstrated that acetic acid is a better preserving agent for sweet pickles than lactic acid. The pH value of the pickle syrup was not a reliable indicator of the preserving power of the acids present. Levine and Fellers (1940) conducted comparative studies and showed that acetic acid was more toxic than either lactic or hydrochloric acid to *Salmonella aertrycke, Saccharomyces cereviseae,* and *Aspergillus niger.* These organisms were inhibited or destroyed at a higher pH value when acetic acid was used as the toxic agent than where lactic or hydrochloric acids were used.

EXPERIMENTAL

Index organisms

The test microorganisms used were *Salmonella aertrycke, Staphylococcus aureus, Phytomonas phaseoli, Bacillus cereus; Saccharomyces cereviseae,* (Lister strain) and *Aspergillus niger.* These microorganisms were selected, not only for their association with food spoilage, but also for their representative value as more or less typical members of certain microbial groups.

Methods

The effect of acetic acid on the total destruction and on the reduction of numbers of bacteria and yeast was determined by a 15-minute contact with acetic acid solutions. The test microorganisms were also subjected to contact with acetic acid solutions containing 5 per cent salt and with acidified 20 per cent sugar syrups. The solutions were prepared as follows: Five milliliters of sterile distilled water were added by pipette to each of a series of sterile tubes. To the first tube, were added 5 ml. of acetic acid of known concentration. After mixing, 5 ml. of this solution were transferred to the next tube. The procedure was repeated until each tube in the series contained 5 ml. of acid solution in decreasing order of concentration, 5 ml. from the last tube having been discarded. One-tenth ml. of a diluted broth culture was added to each tube. The tubes were well agitated and replaced in the rack for 15 minutes. After this contact period a standard quantitative plating was made on each tube. Nutrient agar was used for the bacteria and glucose agar for the yeast.

The same general procedure was followed where acid-sugar syrups and acid-salt brines were used. Five milliliters of sterile double strength salt brine or sugar syrup were added to the first tube so that dilution with an equal volume of acetic acid gave the same strength brine or syrup in this tube as in the others of the series.

Immediately after plating, determination of pH was made on each solution remaining in the tubes. All pH determinations were made electrometrically by means of a Beckman glass electrode pH meter in which the electrode was balanced against a standard cell. Total acidities were calculated, since the acid concentration of the first tube and the subsequent dilutions were known. These calculations were checked occasionally by actual titration with standardized sodium hydroxide.

The mold was cultivated in flasks of 0.5 per cent glucose broth alone and also in glucose broth with 5 per cent salt and with 20 per cent sugar. The broth solutions in each series were acidified with acetic acid. An unacidified control was used in each series also.

The technique employed in the study of *Aspergillus niger* was somewhat different from that used with bacteria and yeast due to the nature of the mold. Sterile 50 ml. portions of 0.5 per cent glucose broth were transferred aseptically to sterile 250 ml. Erlenmeyer flasks. This procedure was used to obviate unequal loss of flask contents through evaporation during sterilization. The sterile sugar- and salt-glucose broths were added to series of flasks in the same way. Varying amounts of acetic acid were added to the flasks to make series of decreasing acid concentrations. The volume of acid added was sufficiently small to affect the surface of the medium but little. Ten milliliters of solution were removed from each flask for pH and total acidity determinations. Acidity was determined by titration with sodium hydroxide with phenolphthalein as the indicator. The remaining 40 ml. gave a maximum surface in each flask for mold growth. A 0.1 ml. water suspension of spores of *Aspergillus niger* was inoculated into each flask. The flasks were held for five days at 30°C. (80°F.) in the absence of light. The dried weight of mold which had developed during the incubation period was determined by filtering and washing the flask contents. The filtrate was saved for pH and total acidity determinations. The mold mats were dried at 100°C. (212°F.) to a constant weight.

Experimental results

Results are presented in tables 1 to 6. The percentage reduction in whole numbers is based on the number of organisms surviving after a 15-minute contact period with similar solution, water, brine, or syrup, that contained no added acetic acid. Thus, in table 1 at a pH 3.3 and a total acidity of 0.15 per cent acetic acid, there was a 98 per cent reduction in the number of cells of *Salmonella aertrycke* surviving from the number obtained when water at pH 6.2 was used as the contact medium. For brine of the same total acidity, 0.15 per cent, the pH was 3.1 and the reduction was 100 per cent. A 20 per cent sucrose syrup that contained 0.15 per cent acetic acid and had a pH of 3.5 caused a reduction from the number of organisms surviving in the unacidified syrup of 99 per cent. Greater numbers of *Salmonella aer-*

trycke survived contact with the unacidified 5 per cent brine and the unacidified 20 per cent syrup than with the water alone. Bushnell (1921) found that broth media containing 4 per cent NaCl

TABLE 1

Effect of different concentrations of acetic acid on the survival of Salmonella aertrycke in water, salt brine, and sugar solutions

Contact period, 15 minutes

TYPE OF SOLUTION	ACIDITY OF SOLUTION		TOTAL COUNT	REDUCTION IN NUMBERS
	pH	Calculated total acidity		
		per cent	*bacteria per ml.*	*per cent*
Water + acetic acid	3.3	0.15	1,870	98
Water + acetic acid	3.4	0.08	4,800	94
Water + acetic acid	3.6	0.04	8,400	90
Water + acetic acid	3.8	0.02	13,800	84
Water + acetic acid	4.0	0.01	24,000	71
Water + acetic acid	4.2	0.005	36,000	57
Water only	6.2	0.0	84,000	
5 per cent brine + acetic acid	3.1	0.15	0	100
5 per cent brine + acetic acid	3.3	0.08	2	99+
5 per cent brine + acetic acid	3.5	0.04	6,000	94
5 per cent brine + acetic acid	3.7	0.02	14,400	85
5 per cent brine + acetic acid	4.0	0.01	48,000	50
5 per cent brine + acetic acid	4.3	0.005	84,000	14
5 per cent brine only	6.1	0.0	96,000	
20 per cent syrup + acetic acid	3.5	0.15	1,320	99
20 per cent syrup + acetic acid	3.7	0.08	7,200	94
20 per cent syrup + acetic acid	4.0	0.04	15,600	87
20 per cent syrup + acetic acid	4.4	0.02	21,600	82
20 per cent syrup + acetic acid	4.7	0.01	72,000	40
20 per cent syrup + acetic acid	5.3	0.005	108,000	10
20 per cent syrup only	7.1	0.0	120,000	

192,000 bacteria were added to each ml. of the solution.

were more favorable for growth of the organisms studied than broth containing one per cent. At the higher total acid concentrations, the brine solution was slightly more effective than the water or the syrup solutions in reducing the number of cells of *Salmonella aertrycke*. However, at the lower acid concentrations

the brine seemed to be less effective than the water of corresponding total acidity, in reducing the number of organisms. The

TABLE 2

Effect of different concentrations of acetic acid on the survival of Staphylococcus aureus in water, salt brine, and sugar solutions

Contact period, 15 minutes

TYPE OF SOLUTION	ACIDITY OF SOLUTION		TOTAL COUNT	REDUCTION IN NUMBERS
	pH	Calculated total acidity		
		per cent	*bacteria per ml.*	*per cent*
Water + acetic acid	3.3	0.17	17	99+
Water + acetic acid	3.4	0.08	114	99
Water + acetic acid	3.6	0.04	260	97
Water + acetic acid	3.8	0.02	2,400	76
Water + acetic acid	4.0	0.01	5,000	50
Water + acetic acid	4.2	0.005	9,200	8
Water + acetic acid	4.4	0.003	10,800	
Water only	6.2	0.0	10,000	
5 per cent brine + acetic acid	3.1	0.17	0	100
5 per cent brine + acetic acid	3.2	0.08	14	99+
5 per cent brine + acetic acid	3.4	0.04	315	96
5 per cent brine + acetic acid	3.6	0.02	1,200	83
5 per cent brine + acetic acid	3.7	0.01	1,500	79
5 per cent brine + acetic acid	4.0	0.005	4,200	42
5 per cent brine + acetic acid	4.2	0.003	6,400	11
5 per cent brine only	6.1	0.0	7,200	
20 per cent syrup + acetic acid	3.5	0.17	0	100
20 per cent syrup + acetic acid	3.7	0.08	1	99+
20 per cent syrup + acetic acid	4.0	0.04	3	99+
20 per cent syrup + acetic acid	4.3	0.02	61	99
20 per cent syrup + acetic acid	4.7	0.01	900	79
20 per cent syrup + acetic acid	5.1	0.005	3,600	14
20 per cent syrup + acetic acid	5.9	0.003	6,000	
20 per cent syrup only	7.1	0.0	4,200	

7,200 bacteria were added to each ml. of the solution.

brine was also less effective than the syrup at equivalent pH but this is not surprising inasmuch as the syrup contained a slightly higher percentage of the acetic acid.

Table 2 shows that plain water reduced the numbers of *Staphy-*

lococcus aureus less than did the unacidified salt or sugar solutions. At the high concentrations of acid, all three solutions were about equally effective in reducing the numbers of added viable bacteria. At pH 4.2 the percentage reduction was approximately the same in the water and in the 5 per cent salt brine solutions, being 8 and 11 per cent, respectively. However, at pH 4.3 in the 20 per cent sugar syrup series, the number of living cells was reduced 99 per cent. In this case some of the reduction may be attributed to the slightly higher acid concentration at this pH value. The syrup permitted the survival of no organisms at pH 3.5 and acidity of 0.17 per cent, whereas at pH 3.3 and the same total acidity, the water solution permitted the survival of some organisms.

Table 3 presents the results obtained on an experiment of a similar nature with *Phytomonas phaseoli*, a plant pathogen. This organism proved to be less resistant to acetic acid than did the other microorganisms. Water and acetic acid at pH 3.4, and brine and syrup at pH 4.0 caused a 100 per cent destruction of the organisms in 15 minutes. In addition, both the brine and the syrup controls with no added acid were more toxic than the water alone. Again, the acidified sugar syrup at any pH caused a greater reduction in numbers than did the brine or water series solution of a corresponding hydrogen-ion concentration. This was true, despite the fact that the unacidified sugar syrup was less toxic to *Phytomonas phaseoli* than the plain salt brine.

Bacillus cereus, an organism closely related to *Bacillus mesentericus* which causes a "ropy" spoilage in many foods, yielded the results in table 4. The unacidified 20 per cent sugar solution seemed to enhance the survival of *Bacillus cereus*, whether as vegetative cells or as spores. But, the addition of acid to the syrup again caused a greater reduction in numbers of surviving organisms than that obtained after contact with brine or water solutions of a corresponding pH value. Although the brine alone was slightly more toxic than the water alone, the latter, when acidified with acetic acid, caused a greater reduction in numbers than the former at the same total acid concentration. If one compares the results on corresponding hydrogen-ion concentration, the difference becomes more marked due, probably, to the

higher acid concentration in the water solution. The same trend, in general, is observed in the case of spores although there are a

TABLE 3

Effect of different concentrations of acetic acid on the survival of Phytomonas phaseoli in water, salt brine, and sugar solutions

Contact period, 15 minutes

TYPE OF SOLUTION	ACIDITY OF SOLUTION		TOTAL COUNT	REDUCTION IN NUMBERS
	pH	Calculated total acidity		
		per cent	*bacteria per ml.*	*per cent*
Water + acetic acid	3.3	0.15	0	100
Water + acetic acid	3.4	0.08	0	100
Water + acetic acid	3.6	0.04	41	99+
Water + acetic acid	3.8	0.02	69	99
Water + acetic acid	4.0	0.01	167	98
Water + acetic acid	4.2	0.005	5,000	50
Water + acetic acid	4.4	0.002	6,000	40
Water only	6.2	0.0	10,000	
5 per cent brine + acetic acid	3.1	0.15	0	100
5 per cent brine + acetic acid	3.3	0.08	0	100
5 per cent brine + acetic acid	3.5	0.04	0	100
5 per cent brine + acetic acid	3.7	0.02	0	100
5 per cent brine + acetic acid	4.0	0.01	0	100
5 per cent brine + acetic acid	4.3	0.005	30	98
5 per cent brine + acetic acid	4.6	0.002	440	68
5 per cent brine only	6.1	0.0	1,380	
20 per cent syrup + acetic acid	3.5	0.15	0	100
20 per cent syrup + acetic acid	3.7	0.08	0	100
20 per cent syrup + acetic acid	4.0	0.04	0	100
20 per cent syrup + acetic acid	4.4	0.02	24	99+
20 per cent syrup + acetic acid	4.7	0.01	900	86
20 per cent syrup + acetic acid	5.3	0.005	4,200	36
20 per cent syrup + acetic acid	6.5	0.002	5,400	18
20 per cent syrup only	7.1	0.0	6,600	

7,200 bacteria were added to each ml. of the solution.

smaller number of organisms involved. Since acetic acid appears to be as effective against spores as against vegetative cells, it is significant that it may be useful for the control of spore-forming bacteria in foods.

Yeasts can generally tolerate higher acidities than most bacteria. Table 5 shows that *Saccharomyces cereviseae* survived contact with acidified water, brine, and syrup solutions at higher total

TABLE 4

Effect of different concentrations of acetic acid on the survival of Bacillus cereus in water, salt brine, and sugar solutions

Contact period, 15 minutes

TYPE OF SOLUTION	ACIDITY OF SOLUTION		TOTAL COUNT; BACTERIA + SPORES	REDUCTION IN NUMBERS	TOTAL COUNT; SPORES ONLY	REDUCTION IN NUMBERS
	pH	Calculated total acidity				
		per cent	*per ml.*	*per cent*	*per ml.*	*per cent*
Water + acetic acid	3.3	0.17	68	79	5	93
Water + acetic acid	3.4	0.08	85	74	17	76
Water + acetic acid	3.6	0.04	94	71	16	77
Water + acetic acid	3.8	0.02	99	70	17	76
Water + acetic acid	4.0	0.01	125	62	21	70
Water + acetic acid	4.2	0.005	150	55	27	61
Water only	6.2	0.0	330		70	
5 per cent brine + acetic acid	3.1	0.17	80	68	4	93
5 per cent brine + acetic acid	3.2	0.08	95	62	13	78
5 per cent brine + acetic acid	3.4	0.04	93	63	15	75
5 per cent brine + acetic acid	3.6	0.02	101	60	16	73
5 per cent brine + acetic acid	3.7	0.01	140	44	25	58
5 per cent brine + acetic acid	4.0	0.005	190	24	26	57
5 per cent brine only	6.1	0.0	250		60	
20 per cent syrup + acetic acid	3.5	0.17	95	87	10	87
20 per cent syrup + acetic acid	3.7	0.08	108	85	17	78
20 per cent syrup + acetic acid	4.0	0.04	114	84	20	75
20 per cent syrup + acetic acid	4.3	0.02	126	83	20	75
20 per cent syrup + acetic acid	4.7	0.01	150	79	22	72
20 per cent syrup + acetic acid	5.1	0.005	210	71	25	68
20 per cent syrup only	7.1	0.0	720		70	

360 bacteria and spores were added to each ml. of the solution.

84 spores were added to each ml. of the solution.

acidities and greater hydrogen-ion concentrations than any of the bacteria studied. The acidity range of the solutions was from 0.0 to 4.73 per cent. A comparison of the water series with the brine series again shows that toxicity is due in part to the acetic

acid itself, without the influence of hydrogen-ion concentration. Although the plain brine was slightly more toxic than the water, the acidified brine caused a smaller reduction in surviving cells

TABLE 5

Effect of different concentrations of acetic acid on the survival of Saccharomyces cerevisiae (Lister strain) in water, salt brine, and sugar solutions

Contact period, 15 minutes

TYPE OF SOLUTION	ACIDITY OF SOLUTION		TOTAL COUNT	REDUCTION IN NUMBERS
	pH	Calculated total acidity		
		per cent	*yeast cells per ml.*	*per cent*
Water + acetic acid	2.5	4.73	0	100
Water + acetic acid	2.7	2.37	78	88
Water + acetic acid	2.8	1.18	300	55
Water + acetic acid	3.0	0.59	420	37
Water + acetic acid	3.1	0.30	470	30
Water + acetic acid	3.3	0.15	500	25
Water only	6.2	0.0	670	
5 per cent brine + acetic acid	2.2	4.73	0	100
5 per cent brine + acetic acid	2.4	2.37	20	97
5 per cent brine + acetic acid	2.6	1.18	370	38
5 per cent brine + acetic acid	2.7	0.59	440	27
5 per cent brine + acetic acid	2.9	0.30	410	32
5 per cent brine + acetic acid	3.0	0.15	450	25
5 per cent brine only	6.1	0.0	600	
20 per cent syrup + acetic acid	2.5	4.73	0	100
20 per cent syrup + acetic acid	2.7	2.37	15	97
20 per cent syrup + acetic acid	2.9	1.18	280	44
20 per cent syrup + acetic acid	3.1	0.59	390	22
20 per cent syrup + acetic acid	3.3	0.30	470	6
20 per cent syrup + acetic acid	3.6	0.15	490	2
20 per cent syrup only	7.1	0.0	500	

900 yeast cells were added to each ml. of the solution.

than did the acidified water at a corresponding pH. Thus, at pH 3.0 the brine caused a reduction of 25 per cent whereas a 15-minute contact period with water at this pH resulted in a reduction of 37 per cent. Yet the water solution at pH 3.0 had a

total acidity of 0.59 per cent while the brine at the same pH had only 0.15 per cent acidity. The greater toxicity of the water solution seems to be due, therefore, to the higher content of acetic

TABLE 6

Effect of different concentrations of acetic acid on the growth of Aspergillus niger in flasks containing glucose broth, glucose broth brine, and glucose broth syrup

MEDIUM	INITIAL ACIDITY		FINAL ACIDITY		DRIED MOLD WEIGHT
	pH	Titratable acidity	pH	Titratable acidity	
		per cent		*per cent*	*mgm.*
Glucose broth	4.1	0.27	4.1	0.27	0
Glucose broth	4.3	0.21	4.3	0.21	17
Glucose broth	4.4	0.16	7.1	0.03*	162
Glucose broth	4.5	0.15	7.6	0.03	148
Glucose broth	4.7	0.10	7.6	0.03	133
Glucose broth	5.2	0.07	7.6	0.03	121
Glucose broth (control)	6.8	0.03	7.6	0.03	128
Glucose broth + 5 per cent NaCl	4.0	0.27	3.9	0.32	0
Glucose broth + 5 per cent NaCl	4.1	0.22	4.2	0.18	33
Glucose broth + 5 per cent NaCl	4.2	0.17	7.5	0.03*	132
Glucose broth + 5 per cent NaCl	4.5	0.12	7.5	0.03	127
Glucose broth + 5 per cent NaCl	4.6	0.10	7.6	0.03	129
Glucose broth + 5 per cent NaCl (control)	6.7	0.03	7.6	0.03	131
Glucose broth + 20 per cent sucrose	4.1	0.27	4.1	0.27	0
Glucose broth + 20 per cent sucrose	4.2	0.22	4.2	0.20	6
Glucose broth + 20 per cent sucrose	4.3	0.20	3.2	0.49	64
Glucose broth + 20 per cent sucrose	4.4	0.16	2.2	2.47	463
Glucose broth + 20 per cent sucrose	4.7	0.11	2.3	2.36	327
Glucose broth + 20 per cent sucrose (control)	6.8	0.03	2.1	3.02	223

The inoculation consisted of 0.1 ml. of a water suspension of *Aspergillus niger* which contained 2000 spores.

* At final pH values of more than 7.0 the apparent acidity represents a blank which is not subtracted in this table.

acid as it could not be due to any difference in the concentration of hydrogen-ions. Bach (1932) stated that generally the hydrogen-ions control antiseptic effect but that the undissociated part of lactic acid is the active factor when pH is such as to be unimportant.

The data in table 6 show the effect of different concentrations of acetic acid on the growth of *Aspergillus niger* in glucose broth, glucose broth with 5 per cent sodium chloride salt, and glucose broth with 20 per cent sucrose. The brine control and the glucose broth control yielded about the same amount of dried mold, 131 and 128 mgm., respectively. However, growth in glucose broth control containing 20 per cent sucrose yielded a dried mold mat weighing 223 grams. This mold growth was sufficient to form 3.02 per cent acid from the sucrose and increase the hydrogen-ion concentration to pH 2.1. The acid produced is calculated as acetic acid for purposes of comparison, although *Aspergillus niger* readily produces citric, oxalic, and gluconic acids when grown in sugar solution. Henrici (1930) states that on prolonged incubation the molds slowly utilize the acids they form thus causing the reaction to return toward neutrality. This is probably what occurred in the acidified broth where sugar was not present and which had mold growth. The acidities were completely utilized from the start and the reaction went beyond the neutral point. In all three series, 0.27 per cent acetic acid, or more, was necessary to prevent the growth of mold. Also, inhibition was about the same in all flasks containing 0.21 and 0.22 per cent acid as 17, 6, and 33 mgm. may be considered as equal insofar as dried mold weight is concerned (see fig. 1). Kirby, Frey, and Atkin (1935) found that the growth of *Aspergillus niger* was inhibited in a bread medium set at pH 3.5 and which contained 0.2 per cent acetic acid.

The heaviest mold mat obtained (see table 6), developed in the 0.5 per cent glucose broth containing 20 per cent sucrose and 0.16 per cent acid with a pH of 4.4. The dried mold weighed 463 mgm. A titratable acidity of 2.47 per cent with pH 2.2 was produced in the medium. In general, the sugar-broth series promoted greater mold development than did either of the other two series at corresponding acidities. The mold growths in the glucose brine series and in the plain glucose series were about the same. At a total acidity of 0.17 per cent, the former yielded 132 mgm. of dried mold whereas the latter at a total acidity of

0.16 per cent yielded 162 mgm. Yet the salted broth had a pH 4.2 which was lower than the pH 4.4 of the 0.16 per cent plain broth solution. This might have inhibited initial growth of the mold sufficiently to account for the difference, as a pH value of 4.2 is close to the inhibiting limit of acetic acid for *Aspergillus niger*. At pH 4.5 the plain glucose broth had an acidity of 0.15

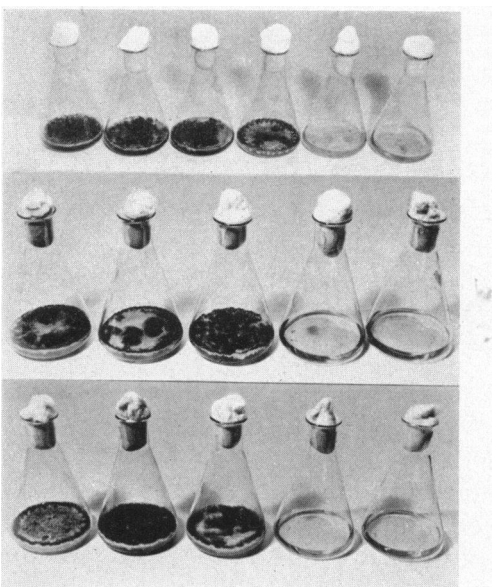

FIG. 1. Growth of *Aspergillus niger* in flasks of glucose broth with added acetic acid (top row); with acetic acid and 5 per cent NaCl (middle row); and with acetic acid and 20 per cent sucrose (bottom row).
Top row: percentage acetic acid, left to right: none, 0.07, 0.10, 0.16, 0.21, 0.27.
Middle row: percentage acetic acid, left to right: none, 0.10, 0.17, 0.23, 0.27.
Bottom row: percentage acetic acid, left to right: none, 0.11, 0.16, 0.22, 0.27.

per cent and yielded 148 mgm. of dried mold. At a pH 4.5 the glucose broth brine had an acidity of 0.12 per cent and yielded 127 mgm. of dried mold. This small difference in mold weight might be attributed to the higher content of acid available for the mold in the plain broth medium, as the hydrogen-ion concentration in this case was not sufficient to cause any inhibition of growth during the first part of the incubation period.

SUMMARY

1. Acetic acid inhibited bacterial growth in almost direct proportion to the amount present. The order of decreasing resistance to acetic acid, either alone, in 5 per cent brine, or in 20 per cent sugar syrup is *Bacillus cereus*, *Salmonella aertrycke*, *Staphylococcus aureus*, and *Phytomonas phaseoli*. Naturally, *Saccharomyces cereviseae* and *Aspergillus niger* were inhibited at a higher acetic acid concentration than the bacteria, with the yeast being the more resistant.

2. At equivalent pH values greater toxicity was usually observed in those tubes containing the greater amount of acid. Apart from the indirect effect in altering the hydrogen-ion concentration, the salt and the sugar aided the acetic acid but little in its toxic effect on bacteria and yeast. Similarly, the added salt and sugar exerted little, if any, effect on the minimum percentage acidity required for total destruction of these organisms.

3. Additional evidence is presented to show that the toxic action of acetic acid on microorganisms is not confined to the hydrogen-ion concentration alone (Levine and Fellers, 1940).

4. When *Aspergillus niger* was inoculated into a series of flasks of glucose broth containing different acetic acid concentrations, growth was inhibited at pH 4.1 and at a total acidity of 0.27 per cent. The addition of either 5 per cent salt or 20 per cent sucrose did not significantly change these limits for growth. The presence of acetic acid in non-toxic amounts actually promoted the development of *Aspergillus niger* by acting as a source of energy.

5. At acetic acid concentrations of 0.10 to 0.17 per cent no inhibition in mold growth resulted from the addition of 5 per cent NaCl. On the other hand the addition of 20 per cent sucrose markedly stimulated growth. Maximum mold growth was obtained in a 20 per cent nutrient sucrose solution at pH 4.4 containing 0.16 per cent acetic acid.

REFERENCES

BACH, D. 1932 The antiseptic action of organic acids on some bacteria. Bull. sci. pharmacol., **39**, 7–23. Chem. Abstracts, **26**, 2210.

BUSHNELL, L. D. 1921 Influence of vacuum upon growth of some aerobic spore-bearing bacteria. J. Bact., **7**, 283–300.

CRUESS, W. V., AND HASCAL, R. 1924 Comparative effects of acetic acid and vinegar bacteria on fermentation. Fruit Products J., **3**, no. 6, 20–21.

FABIAN, F. W., AND WADSWORTH, C. K. 1939 Experimental work on lactic acid in preserving pickles and pickle products. II. Preserving value of acetic and lactic acids in the presence of sucrose. Food Research, **4**, 511–519.

HENRICI, A. T. 1930 Molds, Yeasts, and Actinomycetes. 296 pp., John Wiley and Sons, Inc., New York.

KATAGIRI, H. 1926 The influence of fatty acids and their salts on alcoholic fermentation by living yeast. I. Acetic and formic acids and their sodium, potassium, and ammonium salts. Biochem. J., **20**, 427–436.

KIRBY, G. W., FREY, C. N., AND ATKIN, L. 1935 The growth of bread molds as influenced by acidity. Cereal Chem., **12**, 244–255.

LEVINE, A. S., AND FELLERS, C. R. 1940 Action of acetic acid on food spoilage microorganisms. J. Bact., **39**, 499–515.

PEDERSON, C. S., AND BREED, R. S. 1926 Preservative action in catsup of salt, sugar, benzoate, and acid. N. Y. Agr. Expt. Sta. Bull., 538, 15 pp.

ROWSE, A. E. 1928 A talk on vinegar. Fruit Products J., **8**, no. 3, 15.