SORBIC ACID AS A SELECTIVE AGENT IN CUCUMBER FERMENTATIONS

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THESIS

This is to certify that the

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SORBIC ACID AS A SELECTIVE AGENT IN
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SORBIC ACID AS A SELECTIVE AGENT

IN CUCUMBER FERMENTATIONS

By

Frank Martin Coughlin

AN ABSTRACT

Submitted to the School of Graduate Studies of Michigan
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This investigation was undertaken to determine the effect of sorbic acid (2,4-hexadienoic acid) on the microbiological activity in cucumber fermentations. A total of 48 fermentations carried out in 45 gallon barrels were studied at five different cucumber salting stations in Michigan. The initial NaCl concentration of the brines ranged from 20 to 40° salometer (5.0 to 10.5 percent NaCl). The amount of sorbic acid added to the brines for the two treatments (i.e., 0.05 percent and 0.1 percent) was based on the weight of water used to make up the brine plus the weight of the water in the cucumbers. Barrels containing 0.05 percent and 0.1 percent sorbic acid inhibited the yeast count to less than 100 per ml. as compared to 2×10^5 to 4×10^5 per ml. in the control barrels. Both concentrations of sorbic acid prevented the growth of film-forming yeasts on barrels that were sheltered from direct sunlight. Neither concentration of sorbic acid had any significant effect on the total population of acid-forming bacteria in the 20 and 30° salometer brines. However, the growth rate was slower in lots containing sorbic acid, and the population of acid-forming bacteria was less in the 40° salometer brines. This was more pronounced with the higher concentration of sorbic acid. Sorbic acid had no significant effect on the titratable acidity formed in the lower salometer brines. However, 0.1 percent sorbic acid did reduce the amount even more in the 40° salometer brines. The 0.05 percent sorbic acid, also, resulted in a lower final titratable acidity in the 40° salometer brines.

The principle acid-forming bacteria isolated from all three treatments (i.e., control, 0.05 percent and 0.1 percent sorbic acid) were found to belong to three different species; viz., Pediococcus cerevisiae, Lactobacillus brevis, and Lactobacillus plantarum, in the order of increasing prevalence in the fermentations. Pediococcus cerevisiae was found to occur in appreciable numbers only during the early phase (10 days) of fermentation. L. plantarum was prevalent throughout the fermentation and L. brevis was isolated as frequently as L. plantarum after the first 10 to 15 days. The influence of sorbic acid on the occurrence of the different species of acid-forming bacteria in cucumber fermentations was not considered significant.

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INTRODUCTION

The recent tentative acceptance of sorbic acid by the U. S. Food and Drug Administration as a harmless food preservative has stimulated much research to determine its preservation effect in medium acid and acid foods. Sorbic acid is now used as a mold inhibitor on wrapping material for cheese. This compound has certain qualities that may enable it to replace sodium benzoate as a food preservative in such foods as fruit juices, jams and jellies, fruit sirups, pickles, margarine, and candies. Sorbic acid does not apparently affect color, odor or taste at concentrations necessary for preservation of acid foods. Also, it is less toxic and is a more efficient preservative than sodium benzoate.

There are two principal fermentations involved in cucumber fermentations; viz., the acid fermentation by acid producing bacteria and the yeast fermentation. The acid fermentation is considered desirable and has been found to be directly related to the curing and preservation of cucumbers. The yeast fermentation is believed to be undesirable. Subsurface yeasts produce large quantities of gas which has been found to be related to bloater (hollow cucumbers) formation. Surface yeast metabolize acids and thereby, lower the titratable acidity of the brine. Since work indicates sorbic acid will inhibit yeast at a relatively low pH, below pH 5, and at the same time has no pronounced effect on acid-forming bacteria, it may prove useful in inhibiting the yeast fermentation without interfering with the normal curing of cucumbers.

The purpose of this study was to determine the effect of sorbic acid on the microbiological activity in cucumber fermentations.

LITERATURE REVIEW

Microbiology of Cucumber Fermentations

Lactobacillus plantarum was for some time considered to be the principal species responsible for the acid fermentation of cucumber salt stock (7, 17). Etchells and Jones (17) isolated and studied 49 cultures taken from 20, 30 and 40° salometer (5.0, 8.0 and 10.5 percent NaCl) brines covering a fermentation age range from 3 to 13 days. All 49 isolates, taken from commercial vats of 85 and 720 bushel lots, were found to be *L. plantarum*. Costilow and Fabian (7) isolated 20 cultures from commercial vats ranging in size from 700 to 1100 bushels capacity. Sixteen of the 20 isolates were identified as *L. plantarum*.

More comprehensive studies (5, 30) have demonstrated that there are at least two other species quite active in these fermentations. Pederson and Albury (30) isolated and screened 5,728 cultures of bacteria taken from fermenting cucumber brines of 2 1/2, 5 and 7 1/2 percent salt. They found the principal lactic acid producers to belong to six different species; *viz.*, *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Pediococcus cerevisiae*, *Lactobacillus brevis* and *L. plantarum*, in the order of increasing prevalence in the fermentations. *L. plantarum* was the most common encountered species and was found in nearly all the fermentations. _L. plantarum_ produced a greater quantity of acid and predominated in the later stages of fermentation. However, these results are in some doubt since their experiments were conducted in small laboratory fermentations. Costilow (5) in a study of 848 isolates from barrel lots of fermenting cucumbers under commercial conditions found _Pediococcus cerevisiae_, _L. plantarum_ and _L. brevis_ the predominating acid producers involved. _Pediococcus cerevisiae_ was most prevalent during the first 10 days of fermentation, and the other two species predominated, thereafter.

Many factors have been noted to affect the growth rate and total population of acid-forming bacteria, amount of acid produced and the rate of curing of fermenting cucumbers. The salt concentration of the brine has the greatest effect of these factors (19, 21, 28, 31). The acid-forming bacteria multiply more rapidly, attain higher populations and produce more acid in a shorter time in low salt brines than in high. This, also, results in earlier curing of the cucumbers.

The effect of temperature upon the types of bacteria, total population and titratable acidity has been studied by Pederson and Albury (30) in cucumber brines of 2 1/2, 5 and 7 1/2 percent salt. _Leuconostoc mesenteroides_ and a low acid producing strain of _L. plantarum_ prevailed at temperatures of 45° and 50° F. _Pediococcus cerevisiae_, _L. brevis_ and _L. plantarum_ were more active at 65° F. and above. The maximum fermentation with respect to total titratable acidity and total bacteria count occurred between 75° and 86° F. Curing was completed earlier in brine temperatures which were between 75° and 86° F. However, at 97° F. the initial rate of fermentation was greater than at lower temperatures.

According to Faville and Fabian (23) bacteriophage, antibiotics, and Eh may influence the lactic acid fermentation. The large number of aerobic sporogenic bacteria initially present in the brine were shown to be capable of elaborating a product that is antagonistic towards the acid-forming bacteria. Reduction of the oxidation-reduction potential by these microorganisms was found to increase their salt tolerance. Bacteriophage from the soil or contaminated water also resulted in little or no acid production.

The role and importance of vitamins and amino acids in cucumber fermentations has been investigated (6, 33, 34). In general it was noted that these growth factors were present in sufficient quantities to satisfy the nutrient requirements of the lactic acid bacteria.

The next most prevalent type of fermentation in cucumber brines has been found to be that due to subsurface yeasts. This type of fermentation has been studied in some detail in the major brining areas of the United States (11, 12, 14). Under conditions typical of southern brining areas the major species isolated were <u>Brettanomyces versatilis</u>, <u>Torulopsis caroliniana</u>, <u>Torulopsis holmii</u>, <u>Torulaspora rosei</u>, <u>Hansenula</u>

subpelliculosa, *Zygosaccharomyces halomembranis* and *Zygosaccharomyces globiformis* (14). In northern brining areas the yeast flora was found to be quite similar except *Torulopsis holmii* replaced *Torulopsis caroliniana* as the predominating species during the early fermentation period (12).

It has been shown that the salt concentration of the brine has a greater effect on the time that the yeast fermentation begins and its duration than it does on the yeast population (20). Over a 3 year period, in brines of 20, 30 and 40° salometer, the gaseous fermentation by yeasts was noted to begin earlier and was of shorter duration in 20° brines than in 40° brines. The lactic acid fermentation was believed to have a direct effect on the yeast fermentation. Etchells (20) observed that in low salometer brines an early lactic acid fermentation lowered the pH of the brine rapidly which was favorable for the growth of yeasts. He believed that a vigorous lactic acid fermentation utilized the food material rapidly and limited the duration of the yeast fermentation. Typical yeast fermentations have been found to exist in brines ranging from 20 to 80 percent saturation in respect to salt (36).

Film-forming yeasts occur on brines that are protected from direct sunlight. It has been noted that film-forming yeasts utilize acid and reduce the titratable acidity of the brines (21). Etchells *et al* (11, 13, 15) isolated and studied film-forming yeasts from brined cucumbers in both the northern and southern brining areas. The predominating species found were Debaryomyces membranaefaciens var. Hollandicus and Zygosaccharomyces halomembranis. However, Endomycopsis ohmeri, Endomycopsis ohmeri var. minor and Candida krusei were, also, found in southern brines. Pichia alcoholophilia and Hansenula anomala were found in northern brines of low salt concentrations.

Hydrogen fermentation due to Aerobacter has been shown to occur frequently in southern brining areas (18, 19). Aerobacter cloacae was found to be the major species causing hydrogen fermentations. This fermentation occurred in brine strengths ranging from 20° to 60° salometer. However, it was noted to occur much more frequently and was apparent for a longer time during the fermentation in the higher salt brines than in the lower.

The occurrence of bloaters (hollow cucumbers) in salt stock has been shown to be correlated with gaseous fermentations (26). Over a four year period, involving 28 vats, brines of 20, 40 and 60° salometer were found to have 6.5, 22.9 and 43.6 percent bloaters, respectively (27). Subsurface yeasts and hydrogen fermentations were more active in the higher brine strengths and much larger volumes of gas were evolved from them. Analysis of the gas found inside the hollow cucumbers showed that the proportion of hydrogen and carbon dioxide was about the same as that evolved from the brine surface during fermentation (26).

Sorbic Acid

Gooding (24) discovered that certain alpha, beta-unsaturated fatty acids are good fungistatic agents. Of these, sorbic acid (2,4-hexadienoic acid) was particularly well suited as a fungistatic agent for foods because of its low toxicity, lack of flavor and odor, and effectiveness at low concentrations.

The toxicity of sorbic acid was determined by Deuel, Alfin-Slater, Weil, and Smyth (8) in rats to be approximately one-half that of sodium benzoate. Furthermore, sorbic acid was found to be metabolized in the animal body exactly in the same manner as the natural occurring fatty acid, caproic acid (9).

The mechanism of mold inhibition was determined by Melnick, Luckmann, and Gooding (29). They found that the accumulation of alpha, beta-unsaturated fatty acids in a test medium inhibited the dehydrogenase enzyme system of molds. It was suggested that sorbic acid would not hide an inferior product from the standpoint of mold contamination, because, in the presence of a high mold population, sorbic acid would not retard or impair mold growth.

The minimum threshold for taste and odor of sorbic acid treated cheese was determined by Smith and Rollins (35). When incorporated at concentrations of between 0.2 and 0.5

percent, sorbic acid gave perceptible taste and odor. This was about 4 to 10 times the concentration necessary for fungistatic control.

Emard and Vaughn (10) demonstrated that sorbic acid inhibited catalase positive actinomycetes, bacteria, molds and yeasts at a concentration of 0.12 percent when the initial pH of the medium was between 5.0 and 5.5. They found the type and the initial pH of the media used influenced the inhibitory power of sorbic acid. Sorbic acid was recommended as a selective agent for isolating catalase negative clostridia and lactobacilli.

Phillips and Mundt (32) and Jones and Harper (25) demonstrated that 0.1 percent sorbic acid effectively controls scum yeasts on cucumber brines without inhibiting the lactic acid fermentation to any great extent. Costilow _et al_. (4) found that 0.05 percent sorbic acid at a pH of 5.0 and below inhibited all yeasts common to cucumber fermentations, but had little effect on the lactic acid bacteria. The effect of adding sodium chloride to the media was found to lower the concentration of sorbic acid needed to inhibit yeasts. Conversely, the addition of salt had no appreciable effect on the inhibitory action of sorbic acid toward the lactic acid bacteria. However, Borg _et al_. (1) reported that 0.1 percent sorbic acid in brines containing 9.25 percent salt reduced the population of the acid-forming bacteria to 0.1 that of the controls. Also, the brine acidity was 0.2 percent lactic as compared to 0.5 percent for the controls. Isolates taken from vats containing sorbic acid were found to resemble Pediococcus cerevisiae, L. plantarum, and another species of Lactobacillus. Experimental lots containing sorbic acid had yeast counts less than 100 per ml. The inhibition of the gaseous fermentation resulted in a reduction of bloaters from 60 percent in the controls to less than 5 percent in vats containing sorbic acid.

EXPERIMENTAL PROCEDURE

Fermentations Studied and Methods of Sampling

A total of 48 fermentations were studied during July, August and September of 1954. The experiments were carried on in 45 gallon barrels at five different cucumber salting stations in Michigan. The distribution of the fermentations as to salting stations, the brining treatments used, and the varieties of cucumbers salted may be noted in Table 1.

The cucumbers were graded for size before being placed in the barrels. Size number 3 (1 5/8 to 2 inches in diameter) were used in all fermentations. All fermentations were brined within a 30-day period during July and August of 1954. The cucumbers used for each replication of the three different treatments (i.e., control, 0.05 percent, and 0.1 percent sorbic acid) were grown on the same plot of ground. Duplicate lots of each treatment were used at every salting station with the exception of the 30° brines at station M. Four fermentations of each treatment were used in the 30° brines. The amount of sorbic acid added was calculated from the weight of water used to make up the brine and the water in the cucumber (assuming 90 percent of the weight of cucumber as water).

Samples were taken daily for the first 4 days, every other day up to the 20th day, every 5 days up to the 40th day,

TABLE 1

DISTRIBUTION OF FERMENTATIONS STUDIED, BRINING TREATMENT USED, AND VARIETIES OF CUCUMBERS BRINED

Salting station code No.	Initial brine concen.	Rate of increase of brine concen.	No. of barrels brined	Variety of cucumber used
	° salometer			
LT	35	1 to 2 per week to 60	6	SR-6 and MR-17*
M	20	2 per week to 55	6	SR-6
M	30	2 per week to 55	12	SR-6
M	40	1 per week to 55	6	SR-6
ML**	35***	Held for two weeks, 2 per week to 55	6	National
NL	25	2 per week to 55	6	SR-6
S**	34	1/3 per day to 60	6	MR-17

*Each variety used in the three different treatments.
**Barrels at these stations were under cover.
***Dry salt was not added to compensate for the water in the cucumbers, initial concentration of the brine was 80° salometer and equalized out at about 35° salometer.

and every 10th day, thereafter, up to the 100th day of fermentation. This sampling schedule applies only to the 24 barrels located at station M. The fermentations at the other four salting stations were sampled at less frequent intervals.

A composite brine sample from the barrel was taken by essentially the same technique recommended by Etchells and Jones (16). A stainless steel tube, 3/16-inch in diameter, was placed vertically in the center of the barrel and the sample withdrawn by an aspirator flask. After approximately 500 ml. had run into the flask, a small sample was collected in a sterile vial. The stainless steel tube was sealed at one end and had 3 perforations (2 mm in diameter) spaced 18 inches apart starting at the sealed end.

After the samples were collected they were returned to the laboratory for chemical and microbiological analysis.

Chemical and Microbiological Methods of Analysis

The acidity was determined by titrating with standard NaOH using phenolphthalein as an indicator and calculated as percent lactic acid. The percent NaCl was determined by titrating with standard $AgNO_3$ using dichlorofluorscein as an indicator.

V-8 agar (22) was used for the cultivation and isolation of acid producing bacteria. Dextrose agar plus 0.05 percent yeast extract and acidified with 5 ml. of 5 percent tartaric acid per 100 ml. of medium was used for enumeration of yeasts.

Isolation and Identification of Lactic Acid Bacteria

Three-hundred and twenty-two cultures of acid-forming bacteria were isolated from plates of the highest dilutions of the brine samples. The stock cultures were maintained in trypticase sugar agar (BBL) stabs. They were transferred to micro-inoculum broth (Difco) and incubated for 1 day before inoculating the test media. The bacteria were screened and grouped by determining catalase production, nitrate reduction, gram stain reaction, cell morphology and fermentation of dextrose, sucrose and lactose. Indole-nitrite medium (BBL) was used for nitrate reduction tests (3). Cystine trypticase agar (BBL) was the basal medium for the carbohydrate fermentation studies.

The five groups of bacteria obtained resembled *Pediococcus cerevisiae*, *Lactobacillus plantarum* (nitrate positive), *Lactobacillus plantarum* (nitrate negative), *Lactobacillus brevis*, and a miscellaneous group. Four cultures out of each of the first four groups and all of the cultures in the miscellaneous group were further tested to determine their morphological and physiological properties and identified according to the descriptions given in *Bergey's Manual of Determinative Bacteriology* (2). *Lactobacilli* resembling

L. brevis were tested for gas production by the method recommended by Pederson and Albury (30).

RESULTS

Effect of Sorbic Acid on Yeast Activity in Cucumber Fermentations

The effect of sorbic acid on the yeasts in cucumber fermentations is shown in Fig. I. These data represent averages of duplicate fermentations of each sorbic acid treatment at 20 and 40° salometer and of 4 fermentations per treatment at 30° salometer.

It should be noted that lots containing 0.05 percent and 0.1 percent sorbic acid inhibited the yeast count to less than 100 per ml. after the first four days irregardless of brine strength. Yeast counts of between 2×10^5 and 4×10^5 per ml. were obtained in the control lots of the 20, 30 and 40° salometer brines. In both the 30 and 40° salometer controls, there was an initial decrease in the number of yeasts during the first few days. The yeast fermentation started in the control barrels of all three brining schedules between the 6th and 10th day. The population peak occurred the earliest and the total yeast population was the greatest in the 20° salometer brines. However, the population decreased more rapidly at 20° salometer than at the higher brine strengths.

Both 0.05 and 0.1 percent sorbic acid prevented scum formation on the surface of the brined cucumbers at the two

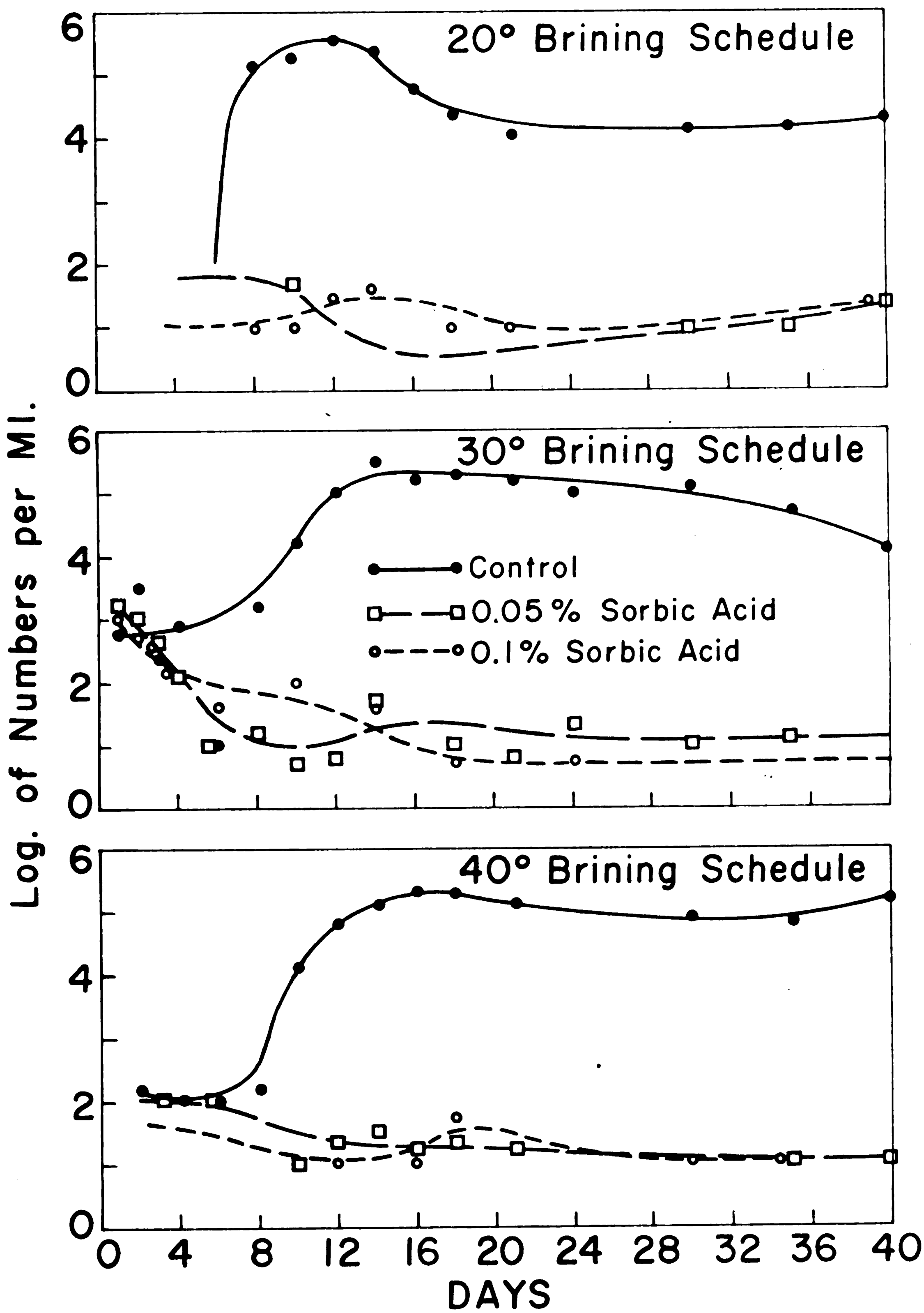

Fig. I. The effect of sorbic acid on yeast activity in cucumber fermentations.

salting stations where the barrels were sheltered from the direct rays of the sun. All the control barrels at these two salting stations had a luxuriant growth of film-forming yeasts.

Effect of Sorbic Acid on the Acid-Forming Bacteria and on Acid Production

The average values of the acid-forming bacteria counts of the 20, 30 and 40° salometer brines at salting station M. may be noted in Fig. II. Neither concentration of sorbic acid had any significant effect on the bacterial populations in the fermentations brined at 20 or 30° salometer. However, the maximum populations attained were somewhat lower in the presence of sorbic acid and the rate of increase was, also, slightly less. In the fermentations brined at 40° salometer, 0.1 percent sorbic acid decreased greatly the growth rate of the acid-forming bacteria. The maximum population was not reached until 12 days after brining as compared to 6 days for the control. The lower concentration of sorbic acid had no significant effect even in the higher salometer brines.

The titratable acidity of these fermentations correlate quite well with the acid-forming bacteria counts (Fig. III). No significant effect of sorbic acid was noted in the low salometer brines. However, 0.1 percent sorbic acid did reduce the amount of acid formed in those fermentations salted

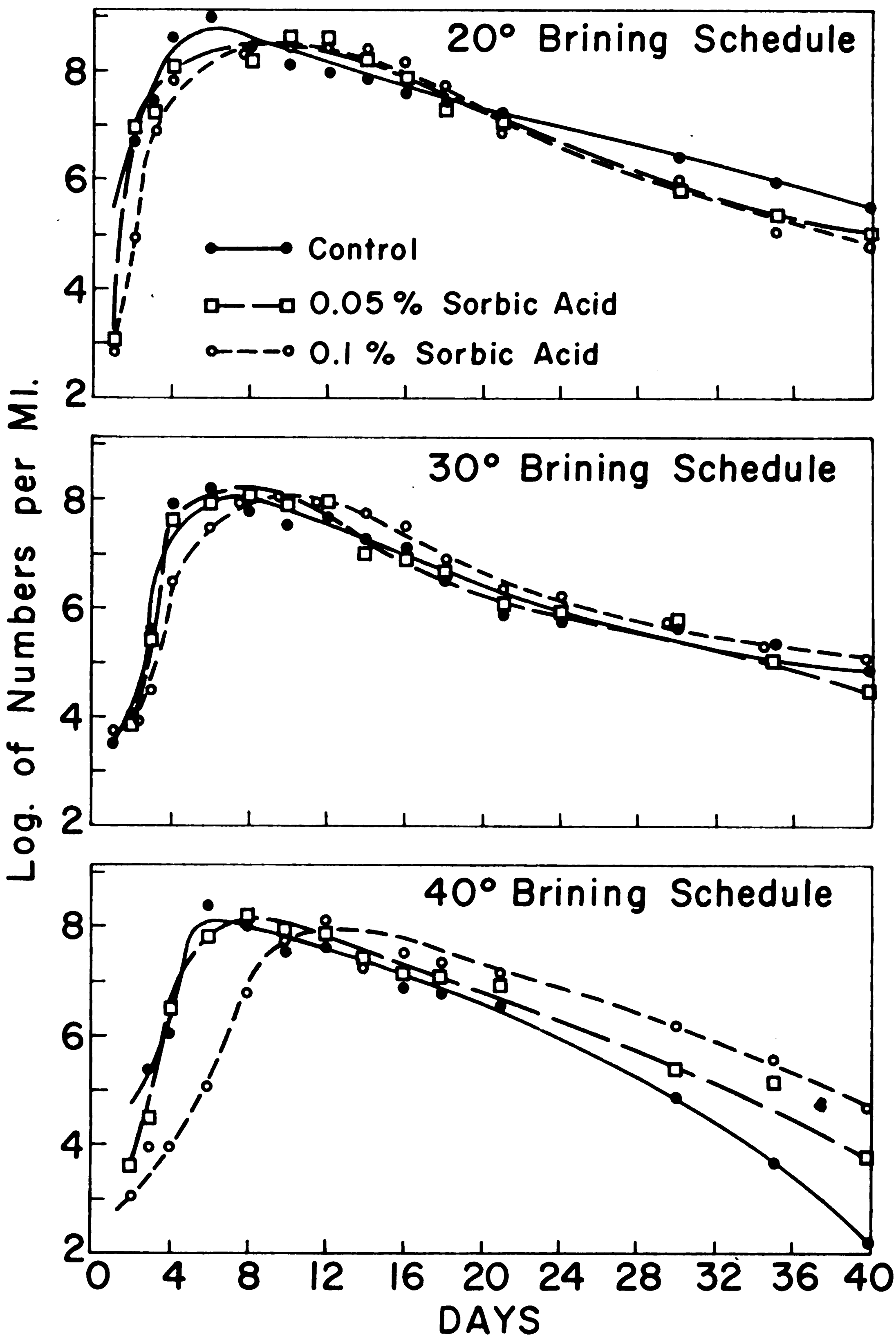

Fig. II. The effect of sorbic acid on the acid-forming bacteria in cucumber fermentations.

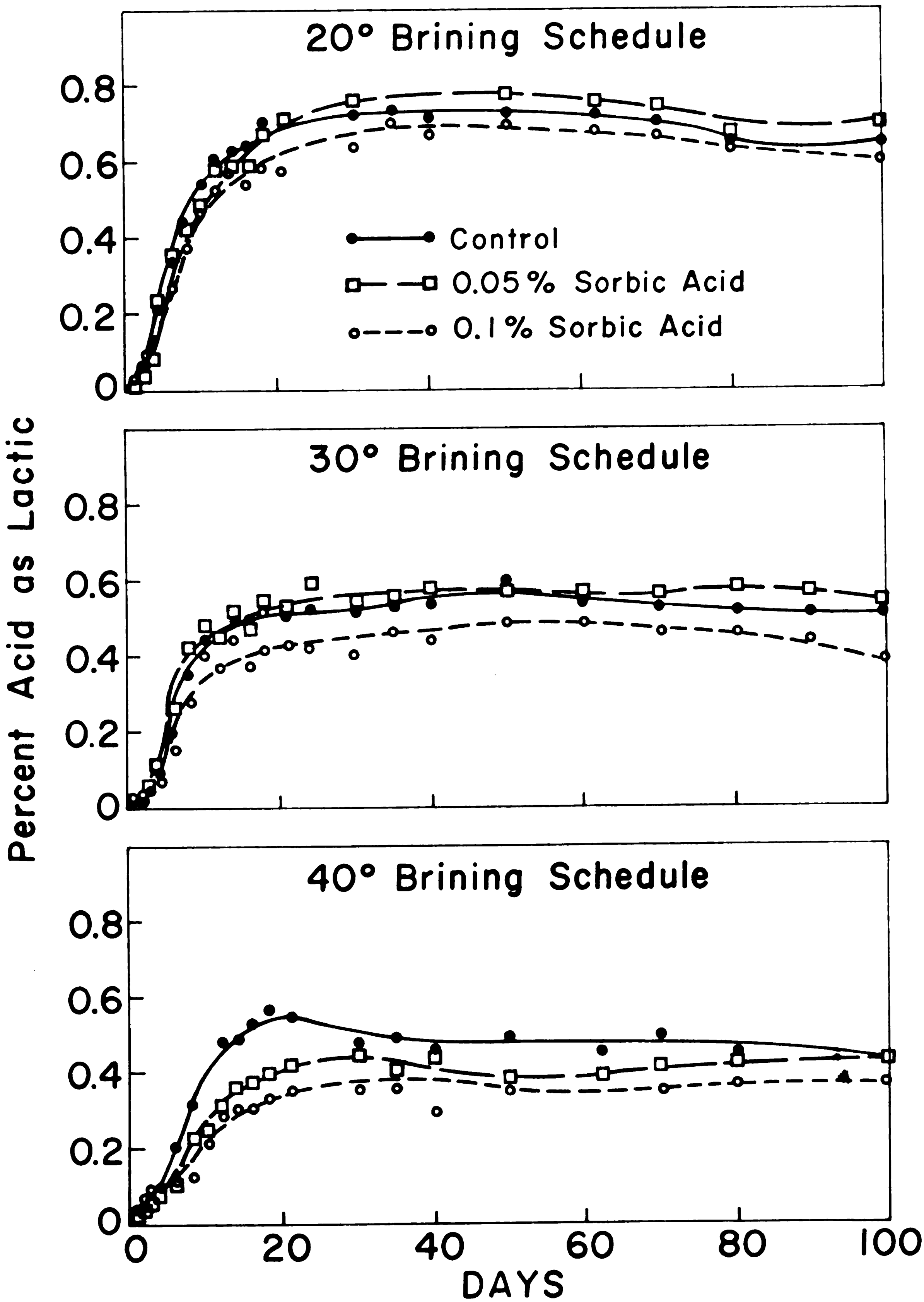

Fig. III. The effect of sorbic acid on acid production at different NaCl concentrations.

at 30° salometer and reduced the amount even more in the 40° salometer brines. The 0.05 percent level of sorbic acid, also, resulted in a lower final titratable acidity in the 40° salometer fermentations.

Influence of Sorbic Acid on the Flora of Acid-Forming Bacteria

A total of 322 cultures of acid-forming bacteria were isolated from 47 cucumber fermentations. Of these cultures, 116 (36.0%) were isolated from control lots, 111 (34.5%) from the 0.05 percent sorbic acid treatment and 95 (29.5%) from the 0.1 percent sorbic acid treatment. The distribution of the cultures as to stations and samples is given in Table 2.

These cultures were identified as follows: *L. plantarum*, 137 (42.5%); *L. brevis*, 106 (32.9%); *Pediococcus cerevisiae*, 61 (18.9%); and a miscellaneous group, 18 (5.6%). Two different groups of *L. plantarum* (nitrate positive and nitrate negative) were isolated. The nitrate positive *L. plantarum* was similar in all other respects to the nitrate negative *L. plantarum* with the exception of its ability to reduce nitrate. Forty-two percent of all *L. plantarum* cultures were found to be nitrate positive.

All 3 major species were isolated from more than one-half of the fermentations (Table 3). Therefore, they were believed to be widespread in fermentations. However, to check on any

TABLE 2

SOURCE OF THE ACID-FORMING BACTERIA CULTURES

Salting station code	No. of fermentations	Initial brine concentration	No. of Samples	No. of isolations
		° salometer		
M	6	20	46	90
M	12	30	38	47
M	6	40	41	97
NL	6	25	13	17
S	6	34	14	21
ML	6	30	17	29
LT	5	35	13	21

TABLE 3

CLASSIFICATION AND DISTRIBUTION OF

ACID-FORMING BACTERIA CULTURES

Species	Cultures isolated		Fermentations isolated from	
	No.	percent	No.	percent
Pediococcus cerevisiae	61	18.9	26	55.3
L. plantarum	137	42.5	39	83.0
L. brevis	106	32.9	29	61.7
Miscellaneous	18	5.7	15	31.9
Total	322	100.0	47	100.0

possible influence of sorbic acid on the occurrence of the various species in cucumber fermentations calculations were made of the percentage of cultures represented by each species from each treatment, and, also, the percentage of fermentations of each treatment from which the different species were isolated (Fig. IV). There was a definite trend that indicated a flora influence of sorbic acid as shown by a decrease in the percentage of L. plantarum and an increase in the percentage of L. brevis with increasing sorbic acid concentrations. This was, also, evident in the distribution among the fermentations. Also, the percentage of fermentation in which Pediococcus cerevisiae was found was less in the sorbic acid treated lots. However, due to the random method of isolating cultures the significance of these results are doubtful. It would be necessary to study a number of fermentations more closely to insure significance of the results.

Pederson and Ward (31) indicated that the salt concentration would influence the acid-forming bacterial flora of cucumber fermentations. Our results failed to confirm this, since the same 3 major species were found in fermentations salted at 20° and at 40° salometer. Also, the relative percentage of cultures of each species isolated were not greatly different (Table 4).

To obtain an estimate of the sequence of the major species occurring in cucumber fermentations, the number of cultures of each species isolated at 5-day intervals during

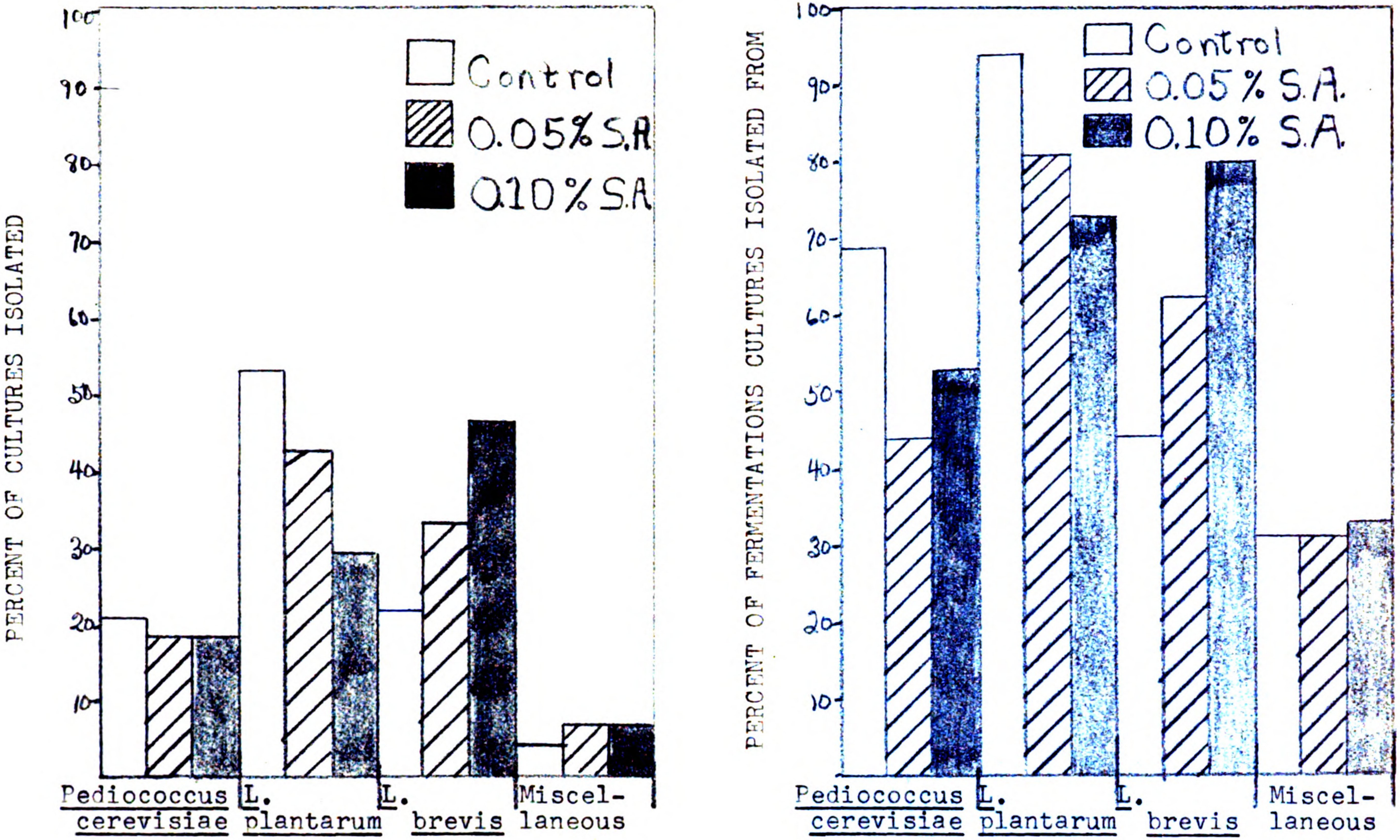

Fig. IV. The influence of sorbic acid on the frequency of isolation of the various species of acid-forming bacteria and on the distribution of the cultures among the fermentations studied.

TABLE 4

THE INFLUENCE OF SALT CONCENTRATION ON THE FLORA OF ACID-FORMING BACTERIA

Species	Salting Schedule			
	20° salometer		40° salometer	
	No.	%	No.	%
Pediococcus cerevisiae	17	18.9	14	14.4
L. plantarum	38	42.2	35	36.0
L. brevis	29	32.2	43	44.3
Miscellaneous	6	6.7	5	5.3
Total	90	100.0	97	100.0

the fermentations were counted and the percentage of the total isolations made at each interval calculated. This estimate of sequence is presented in Fig. V.

These results indicated that *Pediococcus cerevisiae* occurred in appreciable numbers only during the early phase (10 days) of fermentation. *L. plantarum* was prevalent throughout the period studied and *L. brevis* was isolated as frequently as *L. plantarum* after the first 10 to 15 days. It should be noted that strains of *L. plantarum* which failed to reduce nitrate were isolated mostly during the early phase of fermentation and those which did not were largely from the later samples.

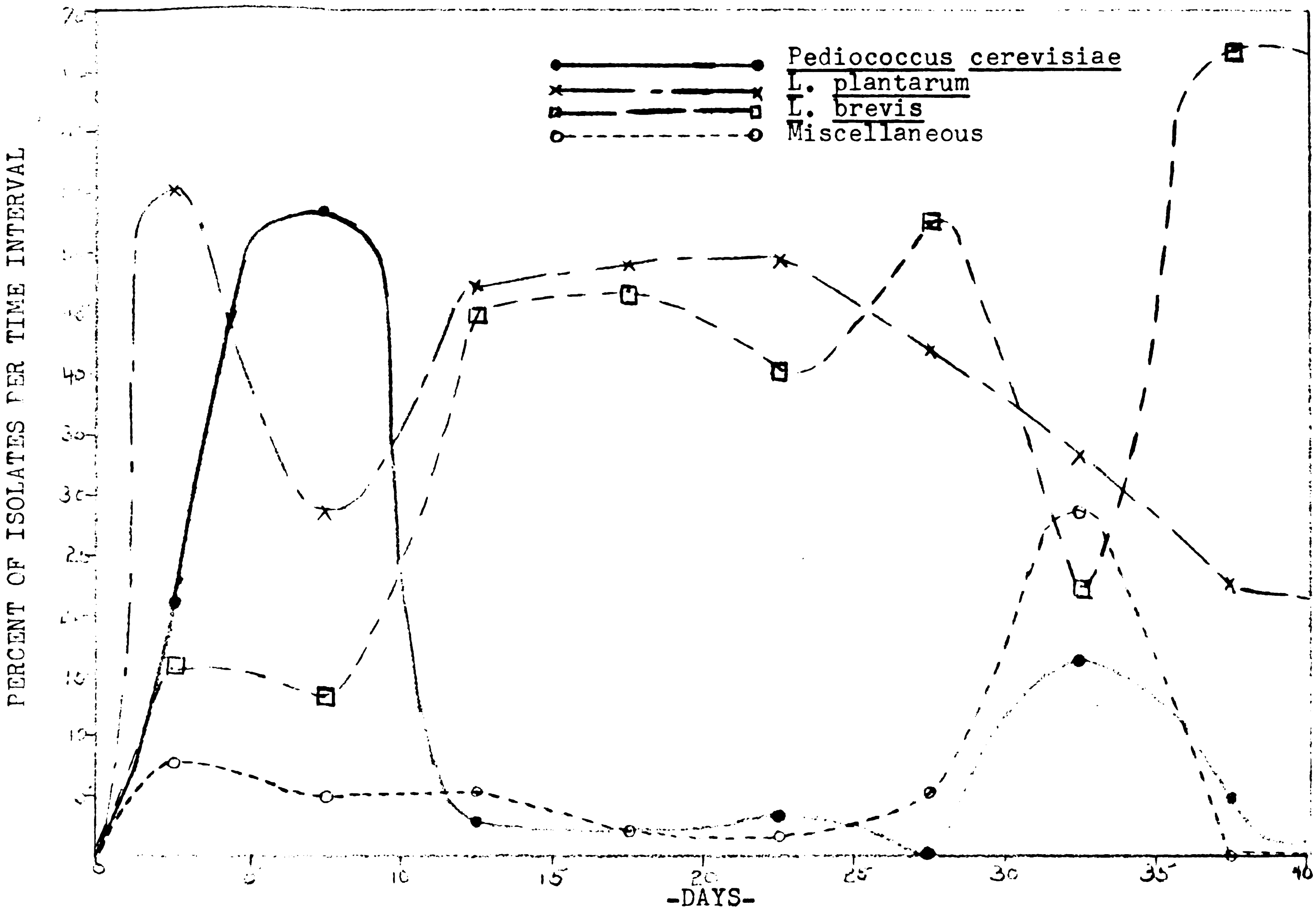

Fig. V. Estimated sequence of the species of acid-forming bacteria in the fermentation of cucumbers.

DISCUSSION

Results from this study demonstrated conclusively that 0.05 percent sorbic acid will inhibit the growth of both film-forming and subsurface yeasts in cucumber fermentations. These results are in agreement with those of Costilow _et al_. (4). Phillips and Mundt (32) noted that concentrations below 0.1 percent would not effectively inhibit surface yeasts. However, they were working with laboratory fermentations and there may have been considerable differences in temperatures and other environmental conditions. Under these conditions one might expect even lower levels of sorbic acid to inhibit yeasts. This has been shown to be true in laboratory media of similar salt concentration and pH levels (4).

The inhibitory effect of 0.1 percent sorbic acid on the population of acid-forming bacteria in this study was not as pronounced as those reported by Borg _et al_. (1). They reported a tenfold reduction over that of the control in 35° salometer brines. This was greater than six times the reduction found in the 40° salometer brines used in this investigation. Their experiments were, also, carried out under commercial conditions, but in a different brining area of this country. Therefore, the climatic and other environmental differences undoubtedly played an important role in determining the influence of sorbic acid on the rate of growth and population of these bacteria. Also, it was not specified in their abstract as to the basis on which the percent sorbic acid added to the brine was calculated.

Results from this study demonstrated that 0.05 percent sorbic acid had little or no effect on the rate of growth of the bacteria and the total population attained in the 20° and 30° salometer brines. These data are in agreement with the results reported by Costilow *et al.* (4) using laboratory test media and at about the same initial pH.

Data presented on the rate of acid production in 40° salometer brines containing 0.1 percent sorbic acid were in agreement with the results of Phillips and Mundt (32); *i.e.*, the acid development was slower in the presence of 0.1 percent sorbic acid than in controls. However, they reported that the acidity of the sorbic acid lots eventually was higher than the highest attained in the controls. This is not too surprising because the acidity of the control never exceeded 0.30 percent lactic acid. It is probable that the film-forming yeasts metabolized some of the acid as it was being formed. Therefore, the control did not provide a good criteria for a comparison of the actual amount of acid formed in the controls. The results of Borg *et al.* (1) were also in disagreement with those reported by Phillips and Mundt (32) and with the findings of this study. They reported that the amount of acid formed in the 0.1 percent sorbic acid treatment was less than one-half that of the control treatments.

Although the 0.1 percent sorbic acid brines had lower acidity in this study at comparable NaCl concentrations the effect of sorbic acid on the acidity produced was not nearly as great.

It was demonstrated in these experiments that 0.05 percent sorbic acid had no apparent effect on the titratable acidity formed in the 20 and 30° salometer brines. These data confirmed the results reported by Costilow _et al_. (4). However, their study on the effect of sorbic acid in combination with NaCl was limited to 6.0 percent NaCl (23° salometer) due to the inability of the test organisms to tolerate NaCl concentrations above this point.

It is apparent from this investigation that 0.05 percent sorbic acid is as effective as 0.1 percent sorbic acid in inhibiting the oxidative and fermentative yeasts found in cucumber fermentations. Also, the lower concentration of sorbic acid did not inhibit the acid fermentation below 30° salometer brine strengths. Results reported by Costilow _et al_. (4) indicate that there is a complementary relationship between NaCl and sorbic acid in the inhibition of yeasts. Thus, it is probable that concentrations of sorbic acid less than 0.05 percent could be used just as effectively for inhibiting the growth of yeasts. This should certainly be investigated. If this should prove to be correct there should be no effect on the acid fermentation in lower brine concentrations. Also, with lower sorbic acid levels, one would expect less effect even in more concentrated salt brines.

SUMMARY

Film-forming and subsurface yeasts were inhibited in cucumber fermentations by both 0.05 and 0.1 percent sorbic acid to less than 100 per ml. The lower concentration of sorbic acid had no significant effect on the bacterial populations in fermentations brined at 20, 30 and 40° salometer. However, the 0.1 percent level decreased greatly the growth rate in the 40° salometer brines.

The sorbic acid treatments had no significant effect on the final titratable acidity in the 20° salometer brines. The 0.1 percent sorbic acid reduced the amount of acid formed in the 30° salometer and reduced the amount even more in the 40° salometer brines. The 0.05 percent sorbic acid level had no appreciable effect on the amount of acid formed in the 30° salometer, but it did result in less acid in the 40° salometer brines than was present in the control lots.

Of 322 cultures isolated from 47 cucumber fermentations, 137 (42.5%) were classified as _L. plantarum_, 106 (32.9%) as _L. brevis_, 61 (18.9%) as _Pediococcus cerevisiae_ and 18 (5.6%) proved to be a miscellaneous group. Sorbic acid did not apparently have any great influence on the occurrence of the three major species. However, there was a definite trend that indicated a switch in the predominating species. Thus,

with increasing sorbic acid levels the frequency of isolation of *L. plantarum* decreased and that of *L. brevis* increased.

The estimated sequence of the species occurring in cucumber fermentations indicated that *Pediococcus cerevisiae* activity was confined to the early phase (10 days) of fermentation. *L. plantarum* was prevalent throughout the fermentation and *L. brevis* was isolated as frequently as *L. plantarum* after the first 10 to 15 days.

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