INFLUENCE OF SUGAR, AMINO ACIDS, AND CORN STEEP LIQUOR ON LACTIC ACID PRODUCTION AND THE MICROBIOLOGIC ACTIVITY OF CUCUMBER FERMENTATION

By

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AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and Applied Sciences
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Microbiology and Public Health

1956

Approved, Feb. 14, 1956

W. J. Stafseth

Studies were made in 1953 and 1954 under semicommercial and laboratory conditions of the influence of the addition of sugar, addition of cystine and tryptophane, and addition of corn steep liquor on the production of lactic acid, the microbiologic activity and the availability of certain vitamins and amino acids in cucumber fermentations.

Under the conditions of these experiments, acid production was not increased by the addition of sugar or amino acids. Under ideal conditions Lactobacillus plantarum was capable of producing only so much lactic acid irrespective of the amount of sugar present in the brine. Corn steep liquor enhanced acid formation. It also increased faster and greater utilization of sugars in the brine.

No significant effect of added sugar or added cystine and tryptophane on the total and acid-forming bacterial populations and yeasts was observed. Addition of sugar on the 7th day, however, caused a rapid increase in the yeast population which was followed by a rapid decline when the sugars in the brine were depleted. Addition of corn steep liquor stimulated the bacterial flora resulting in rather high numbers observed under both semicommercial and laboratory conditions. Conversely yeasts were lower, especially after the active fermentation had ceased.

With the exception of tryptophane, the vitamins and amino acids required for acid production by L. plantarum,

were not affected by the different treatments. Addition of cystine and tryptophane, or corn steep liquor showed that tryptophane was not likely to be a limiting factor for L. plantarum.

The possibility that corn steep liquor might contain a substance or substances, other than those examined, which stimulated the acid-forming bacteria when its concentration was depleted in the brine, was indicated.

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ACKNOWLEDGMENTS

The author wishes to thank Dr. F. W. Fabian for his guidance in various aspects of this study. Acknowledgments are also made to Dr. R. N. Costilow and Dr. S. Rosen for their advice in demonstrating different techniques. The cooperation of the H. W. Madison Co. at Mason, Michigan, is greatly appreciated.

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INTRODUCTION

The commercial manufacture of cucumber pickles generally involves three principal processes. The first is the production of salt stock by salting fresh cucumbers in sodium chloride brines of suitable concentrations. The second is the processing or de-salting of the salt stock to withdraw the excess salt, and the third is the finishing of the de-salted stock into the many kinds of dill, sweet, and kosher pickles and pickle products.

After the cucumbers are harvested at the proper stage of maturity they are inspected for defects and graded for size. They are then placed in vats and covered with brine ranging in concentration from 22° to 40° salometer depending on climate and other environmental conditions.

In general, there are two methods for adding brine to cucumbers, although there are many variations of these methods. In one method the initial brine concentration is 30° salometer (nearly 8.0 per cent NaCl); and sufficient salt is added to raise the brine two degrees salometer each week until a final reading of 50° (13.1 per cent NaCl) is reached; and one degree each week until 60° salometer or higher is reached. In the second procedure a brine containing 40° salometer (10.6 per cent NaCl) is used, and the salt concentration of the brine

is increased two degrees each week until it reaches 50°, and thereafter one degree each week until a final reading of 60° salometer or higher is obtained.

When the cucumbers are surrounded by the brine they lose water by osmosis. Dissolved in this water are sugars, soluble proteins, vitamins, minerals, and other substances which are used as food by the lactic acid bacteria and many other organisms found in the brine. As the dissolved nutrients are withdrawn, there is a penetration of the salt into the cucumbers. Thus the brine is diluted, and under these circumstances, the salt concentration of the brine, unless corrected, may become so reduced that spoilage type of microorganisms may grow and result in an undesirable fermentation. Consequently, numerous investigations have stressed the importance of carefully controlled salt concentrations of the brine.

In the normal fermentation that takes place in cucumber stocks, the fermentable carbohydrates, largely reducing sugars, are almost completely converted into organic acids. The principal acid formed is lactic. Most commercial manufacturers consider the development of an appreciable brine acidity necessary for curing cucumbers and for maintaining a desirable prime color during the subsequent storage of the stock until it is used. Furthermore, it is the opinion of many that the acid formation should start promptly after brining and be maintained at a rapid rate to prevent the growth of undesirable organisms.

As Lactobacillus plantarum is the organism mainly responsible for the acid formation (7, 20), rapid utilization of sugars by it is desirable. Several investigations have suggested the addition of sugar to the brine in order to accelerate the acid formation, or favor the production of greater amounts of it.

In this work, semicommercial and laboratory fermentations have been studied with the object of investigating the effect of different treatments of cucumber salt stocks on the formation of acid by the lactic acid bacteria.

REVIEW OF LITERATURE

Influence of Sugar on Lactic Acid Formation

Several investigators have recommended the addition of sugar to salt stock cucumber brines with the object of accelerating and producing more lactic acid. Other workers however, do not favor it. The following is a review of the literature found on the subject.

As early as 1899 Aderhold (1) claimed that the addition of 0.05 to 0.1 per cent dextrose to cucumber brine resulted in faster and more acid production than in the case where sugar was not added.

In 1920 (36) and 1922 (37) LeFevre showed that the addition of a readily fermentable sugar to pickle brine at the beginning of the salting process resulted in more active fermentation. Consequently higher and more prolonged acidity occurred.

In 1929 Joslyn (34) reported that the use of two to five pounds of dextrose per barrel of dill pickles accelerated the fermentation and decreased spoilage by producing more lactic acid.

In 1932 Fabian et al. (22) investigated the addition of destrose or sucrose when the salt concentration was 66° salometer and after the active fermentation had ceased. They indicated that the added sugar seemed to be beneficial

when pickles are to be stored for any length of time. There were sufficient acid-producing bacteria to cause an increase in acidity. In a similar experiment, carried out in the laboratory, addition of one per cent sucrose increased the acidity from 0.30 to 0.85 per cent.

In 1935 Fabian and Wickerham (25) studied the curing process in the production of genuine dill pickles. They showed that the addition of cane sugar at the beginning of the fermentation accelerated the curing process and decreased spoilage of pickles.

In 1937 Fellers et al. (28) conducted experiments on cucumber salt stock and other pickled vegetables. Dextrose, in concentrations of one and two per cent of the weight of the vegetable, was added before fermentation started, to brined vegetables, ranging from 5 to 15 per cent salt. The addition of sugar materially increased the titratable acidity which, in turn, decreased bacterial spoilage and improved the quality and texture of the pickled vegetable. The most striking results were obtained with dill and salt stock cucumber pickles.

In the same year Campbell (6) also recommended the addition of one to two per cent glucose or any other cheap fermentable sugar in the curing of salt stock if it is desired to produce a higher percentage of acidity.

In 1938 Fabian and Johnson (24) indicated that the influence of sugar addition to dill pickles or cucumber salt

stock depended upon two factors: the amount of sugar already present in the cucumber, and the bacterial flora of the brine.

In 1939 Fellers (27) investigated the addition of dextrose to fermenting genuine dill pickles and cucumber salt stock. For three pickling seasons, the results showed that the addition of sugar accelerated the fermentation. It also resulted in a slightly increased final acidity and improved the keeping quality of the product. Other advantages cited for addition of sugar were better control of slippery pickles and bloaters. The added sugar might have been beneficial in supplementing low sugar cucumbers.

However, Jones et al. (33) observed no beneficial results from the sugar treatment in the manufacturing of genuine dill pickles. When three and one half pounds of sugar was added at the start of fermentation, the amount of lactic acid increased over the control lots by 0.1 per cent. When the same amount of sucrose was added on the 7th day of fermentation, less acid was observed. In both cases, however, the variations were not much greater than those that would normally be expected with the same treatment.

In 1941 Veldhuis and co-workers (55) showed that in four curing seasons, the addition of sugar to salt stock or dill brines at the start of fermentation, or after 10 days, or in small amounts at short intervals throughout the fermentation, caused no appreciable change in acidity as compared with the control.

Influence of Sugar on the Microbial Population of Cucumber Fermentation

Many workers have investigated the influence of sugar addition on the bacterial flora of cucumber fermentation.

In 1932 Fabian *et al.* (22) studied the bacteriologic changes when the sugar was added to cucumber salt stock at 66° salometer. The results showed that the number of acid-producing bacteria immediately increased after sugar addition despite the high salt concentration.

In 1935 Fabian and Wickerham (25) showed that the addition of sugar at the beginning of fermentation increased the number of weak acid-producing bacteria. The low pH levels in the early stages of fermentation enhanced the growth of the acid-forming bacteria and enabled them to outgrow any spoilage types of microorganisms.

In 1937 Fabian (21) explained that the beneficial effect of sugar addition was due to presence of an adequate supply of energy source for all organisms in the brine, which enhanced acid production and protected the pickles from spoilage.

In addition, the studies of Fellers *et al.* in 1937 (28) and those of Fellers in 1939 (27) showed that the sugar stimulated lactobacilli and consequently increased acidity.

Also, the results of Fabian and Johnson (24) agreed with those of Fellers (27, 28), i.e., salt inhibited

the growth of pectin decomposers whereas sugar stimulated the acid-producing organisms.

In 1940 Jones et al. (33) reported that by adding sucrose to fermenting dills, the population of acid-forming bacteria markedly increased with no appreciable rise in final acidity. Also, the yeast population was somewhat higher when sugar was added.

Furthermore, Veldhuis et al. (55) indicated that the addition of sugar to brines at the start of the curing process of salt stock or dills, resulted in a condition that favored an increase in microbial population. This increase appeared in some cases to be only that of the acid-forming bacteria, whereas in other cases, only yeasts generally increased. Still in other cases, greater numbers of both types of organisms were observed. There was no increase in acid production, however, even when the acid-producing bacteria showed a significant increase in numbers as a result of sugar addition. When the experiment was carried out on a commercial basis, the addition of sugar did not affect the numbers of the acid-forming bacteria, whereas yeasts increased rapidly.

Corn Steep Liquor

Corn steep liquor or corn steep water is a nutrient extract obtained during the manufacture of starch and other corn products. During the processing of corn, it is steeped for

approximately two days in warm water containing a small quantity of sulfur dioxide to extract the soluble materials. The steep water is concentrated to approximately 12 Baumé. Batches of corn steep liquor, made by several manufacturers, have different compositions. In general, corn steep liquor contains, on a dry weight basis, 12 to 27 per cent lactic acid, 7.4 to 7.8 per cent total nitrogen, 2.6 to 3.3 per cent amino nitrogen, 1.5 to 14 per cent reducing sugars (calculated as glucose), and 18 to 20 per cent ash (2). In addition, corn steep liquor appears to contain adequate quantities of KH_2PO_4 and $MgSO_4 \cdot 7H_2O$ (29).

Corn steep liquor has been incorporated in several media used for the industrial manufacture of various organic acids and antibiotics.

Influence of Corn Steep Liquor on Cucumber Fermentation

Since corn steep liquor is a cheap industrial waste product, it could be used, if beneficial, on a commercial basis in cucumber fermentations. Very little information, however, is available on its use for such a purpose.

A patent by Pollak (43) in 1941, recommended the addition of one ounce of corn steep liquor per gallon of brine for making genuine dill pickles. Its advantages may be summarized as follows:

(1) The lactic acid formation started earlier and reached a higher maximum when compared with control batches. For example, when corn steep liquor was added at the start of the fermentation of genuine dill pickles, the acidity reached 0.7 per cent after four days. Without steep water, the same acidity level was reached in 8 days. In addition, after 30 days of fermentation, the acidity reached 1.4 per cent in the cases where corn steep liquor was added, whereas in control batches it decreased to 0.6 per cent. Furthermore, the treatment of cucumber fermentations with corn steep liquor showed more acid formation when compared with the addition of sugar.

(2) The formation of surface yeast scum did not occur in contrast to parallel batches which were not treated. Consequently, spoilage did not occur in the barrels which were treated with corn steep liquor.

(3) Addition of corn steep liquor improved the quality of the product and eliminated all faulty fermentations.

Bacterial Changes During Fermentation

In 1913 Rahn (44) studied the fermentation of cucumber under commercial conditions in Michigan and reported that the total number of bacteria at the beginning of fermentation was

about 350 per ml, but reached 200,000,000 per ml during active fermentation.

In 1932 Fabian et al. (22) showed an increase in the total number of bacteria during the first 24 hours after the cucumbers were placed in either 30° or 40° salometer brine. A gradual decrease then occurred when the brine concentration increased to 50° salometer. While the total number of bacteria decreased due to suppression of the peptonizing bacteria, there was a gradual increase in the acid-producing organisms. Furthermore, the number of the acid-producing bacteria was greater and reached a maximum sooner in 30° salometer brine than in the 40°. They claimed that the following factors influenced the number of the acid-producing bacteria: concentration of salt, presence of available food, and acidity produced by the bacteria themselves.

In 1943 results obtained by Etchells et al. (19) under commercial conditions agreed with those of Fabian et al. (22). When comparing initial brine concentrations of 20°, 40° and 60° salometer, they reported that the highest populations occurred in the 20° brines. Also, fermentation at 20° brine started earlier. However, the duration of fermentation was shorter than in the case of 40° brine. When the brine salt concentration was 60° salometer, the acid-forming organisms were markedly inhibited and, therefore, the population progressively decreased.

In 1953 Rosen and Fabian (45) investigated the population changes in cucumber salt stock prepared in five-gallon crocks with an initial salt concentration of 30° salometer. They showed that the acid-forming bacteria did not appear until 24 hours after the start of fermentation. Then, they persisted in large numbers for about one week, after which there was a gradual decline.

In the same year Costilow and Fabian (8) found that the acid-forming bacteria, principally Lactobacillus plantarum, grew rapidly within 24 hours after brining under both commercial and laboratory conditions. The organisms attained their maximum population in about three to five days, declined rapidly for the next five days, and then continued to decline at a slower rate for the rest of the examination period which was over 50 days.

Yeast Changes During Fermentation

Different environmental conditions affect the yeast population present during cucumber fermentations. For example, it has been shown (19) that the population of yeasts reached a higher maximum at 60° brines than at 40° or 20°. Furthermore, the yeast fermentation started earlier at 20° than at 40° or 60° brines.

However, Etchells et al. (17) indicated that the different salt concentrations did not materially influence the number of yeasts found in the brine.

In 1953 Costilow and Fabian (8) found that, under commercial conditions (10 tanks examined), the yeast population declined for the first three to five days before initiating rapid growth. The peak populations were reached between 10 and 20 days after brining. A steady decline in numbers was noted thereafter. In the laboratory experiments however, the yeast numbers were relatively high throughout the period of examination. Yet Rosen and Fabian (45), in experiments carried out in five-gallon crocks at 30° salometer, did not observe the decrease in yeast population at the beginning of fermentation. The yeasts which were present initially gradually increased in number and reached a peak in about 8 days, after which a gradual decline occurred.

Coliform Changes During Fermentation

Population changes of the coliform organisms during fermentation of cucumber salt stocks are influenced by several factors. When studying the effect of different salt concentrations on these organisms Etchells et al. (19) found that in 20° brines the rapid onset of acid fermentation cut short the activity of Aerobacter because they were unable to tolerate the increasing acid content of the brine. Their numbers declined rapidly after two days and reached a level of 1000 cells per ml on the 4th day of fermentation. In 40° brine the Aerobacter fermentation started promptly and remained

active for about five days with counts above 1000 per ml. On the 8th day a sharp decline occurred and the count was 1000 organisms per ml. In 60° initial brine concentration a different picture was observed. The number of Aerobacter organisms constantly declined during the first 6 days, and remained more or less stationary for a short interval. Then a fermentation started, covering an active period from about the 11th to the 18th day. A gradual decrease was observed thereafter.

In another study Etchells et al. (17) reported typical hydrogen fermentation at 60° salometer salt treatment. At 40° brine, hydrogen fermentation may or may not result, whereas in the 20° treatment it was generally absent. In general, the fermentation at the higher salt concentrations resulted in larger quantities of evolved gas.

On the other hand, when Costilow et al. (8) studied the Aerobacter fermentation under laboratory and commercial conditions in Michigan no significant activity of the coliform bacteria was noted. They indicated that coliforms, when entering the brines on the cucumbers, must have found the conditions unfavorable for their growth and consequently died.

The results by Rosen et al. (45) obtained under laboratory conditions, agree with those of Costilow et al. (8). Aerobacter and related organisms at the start of fermentation grew rapidly for 24 hours, then declined sharply for the rest of the examination period.

Biochemical Changes in Fermenting Cucumbers

Early work by Rahn (44) showed that, under commercial conditions, the acid content of cucumber brines varied from 0.6 to 1.2 per cent at the end of active fermentation.

In 1916 Brown (4) observed the formation of lactic and acetic acids in the ratio of 2 to 1. Other acids, e.g., propionic, butyric, and benzoic, occurred in traces.

In 1926 Tanner (50) claimed that brine acidity resulted from volatile acids rather than lactic.

Today it is well known that several factors affect acid formation during cucumber fermentation.

Bacteriophage from soil, antibiotics produced by microorganisms and low Eh value due to the growth of certain types of organisms may result in little or no lactic acid formation (26).

The initial salt concentration of brines also have been mentioned as an influencing factor. LeFevre (36, 37) and Jones (30) showed more acid formation at high salt concentration when compared with weaker brines. For example, the average acidity was 0.8 per cent when the initial salt concentration varied from 5 to 7 per cent, whereas acidity reached 2.4 per cent with initial salt concentration of 8 to 10 per cent.

However, Fabian et al. (22) reported that the titratable acidity was greater and reached a maximum sooner in the 30° than in the 40° salometer brine.

Results by Etchells and Jones (19) and Jones and Etchells (32) showed that the titratable acidity reached a maximum of about 0.7 per cent in 8 to 9 days when the initial brine concentration was 20° salometer. In 40° salometer brine, a maximum acidity of only 0.4 per cent occurred after a period of 12 to 13 days.

In 1949 and 1950 Pederson et al. (40, 41) investigated the effect of the initial brine concentration and temperature on acid formation. Their results showed more acid production in the lower brine concentrations than in the higher concentrations. At 45°F, fermentation was limited and the curing process was slow. The initial rate of acid formation was greater at 97°F, but the extent of fermentation was usually greater at 75° and 86°F.

Microbiology of Cucumber Fermentation

It is well known that the character of any cucumber fermentation is dependent upon the type and number of organisms present and their effect during the course of fermentation.

Acid-forming organisms during cucumber fermentation. The first systematic study of the microflora of cucumber fermentations was done by Fabian et al. (22). They showed that the flora consisted chiefly of weak acid formers.

In 1933 Wustenfeld (57) divided the lactic acid bacteria found in cucumber fermentation into the genera: Bacillus, Streptococcus, and Pedecoccus.

In 1935 Vahlteich et al. (53) were the first to conduct bacteriologic studies of cucumber fermentation under commercial conditions. They believed that the formation of acid during fermentation was due to Lactobacillus cucumeris and two species of the genus Leuconostoc.

Several other workers (7, 8, 20) considered Lactobacillus plantarum (Orla Jensen), Bergey et al., the predominantly active organism in salt stock cucumber fermentation.

However, studies by Pederson (40, 41) showed that one or more of the following organisms might also play an active role in these fermentations: Leuconostoc mesenteroides, Streptococcus feacalis, Pediococcus cerevisiae, and Lactobacillus brevis. However, his studies were conducted under laboratory conditions and at a low salt concentration and, therefore, are not typical of commercial cucumber fermentations.

Yeast fermentation. A secondary fermentation which occurs in fermenting cucumbers is caused by the action of subsurface yeasts. Several workers have mentioned true yeasts in connection with cucumber fermentation. Recently population studies (8, 12, 13, 16, 45) showed that a part of the typical fermentation of cucumbers was brought about by yeasts, such as Torulopsis caroliniana, Torulopsis rosei, Torulopsis

holmii, Brettanomyces versatilis, Hansenula subpelliculosa, and Zygosaccharomyces.

Furthermore, film producing yeasts might grow on top of brines especially when protected from the sun light (14, 15, 22). Etchells et al. (14, 15) have identified Debaromyces membranaefaciens var. Hollandicus, Zygosaccharomyces Halomembranis, Hansenula anomala, Endomycopsis ohmeri, Candida krusei, and Pichia alcoholophilia, as species of scum yeast, under commercial conditions, and in salt concentration ranging from 5 to 19 per cent. Endomycopsis ohmeri var. minor was associated with film formation under laboratory conditions.

Gaseous fermentation. Gaseous fermentations have been frequently observed in cucumber salt stock in the southern areas of the United States.

In 1939 Veldhuis and Etchells (54) reported a definite correlation between CO_2 production and the presence of typical yeast fermentation. In 40° and 60° salometer brines, two distinct phases of gas evolution were observed (17). The first phase was brought about by the Aerobacter group with gases evolved, similar in composition with respect to H_2 and CO_2. The second phase was brought about by yeasts, and the gases evolved considered chiefly of CO_2.

Recent studies showed that Aerobacter, especially A. cloacae, was the major species that caused the formation of gas (17, 19, 45).

Bloater Formation

In commercial production of cucumber salt stock, formation of bloaters (or hollow cucumbers) is considered undesirable. Several investigators have carried out extensive studies on the reasons for production of bloaters. In 1939 Veldhuis and Etchells (54) and Etchells et al. (17) found marked similarity between the gases formed in hollow cucumbers collected from the top and interior of the vats. In 1941 Etchells et al. (18) indicated that the chemical analyses in sweet pickle stock showed the production of CO_2 and alcohol, whereas non-volatile acids were absent. Therefore, yeasts were considered responsible for the gaseous fermentation resulting in the production of bloaters. Other studies carried out by Etchells and Jones in 1943 (19) and Etchells and Bell in 1950 (13) incriminated Aerobacter and yeasts in the formation of bloaters.

It would thus appear, that Aerobacter and yeasts are in general associated with the production of bloaters in cucumber fermentations.

Other studies dealing with the addition of sugar to cucumber fermentations have indicated marked increase in the percentage bloaters formed as a result of this treatment (18, 55).

Biotin, Niacin, and Pantothenic Acid in Cucumber Fermentation

It is well known that the lactic acid group of organisms is responsible for the acid fermentation in cucumber salt stocks. L. plantarum is considered by many workers to be one of the most active organisms during cucumber fermentation. This species is quite fastidious in its nutritional requirements and is used in many microbiological assays for vitamins and amino acids. Since biotin, niacin, and pantothenic acid were shown to be required by many lactic acid bacteria (42), they were selected for this investigation. The following review of literature will deal only with these vitamins.

Until recently, practically no information could be found as to the concentrations of biotin, niacin, and pantothenic acid in fresh cucumbers and cucumber pickles. The nutritional data published in 1950 by H. J. Heinz Company (56) indicated 0.2 mg per 100 gm of nicotinic acid in cucumbers. In 1953 Rosen and Fabian (45) studied the amounts of biotin, niacin, and pantothenic acid available for the lactic acid bacteria during cucumber fermentation. When comparing the vitamin requirements of 10 isolates of L. plantarum from cucumber fermentations with the well known L. arabinosus 17-5, they established its need for biotin, niacin and pantothenic acid. Furthermore, they analyzed samples of cucumber juice obtained from 6 different varieties of cucumbers, and reported that biotin concentration varied from 5.2 to 33

mmcg/ml; niacin from 1.83 to 5.05 mcg/ml; whereas pantothenic acid showed a concentration of 1.05 to 2.42 mcg/ml. In addition, they investigated the effect of different brine microorganisms (9 isolates of A. cloacae and 9 isolates of yeast) on the utilization or synthesis of these vitamins. Aerobacter cloacae markedly lowered the biotin content of the cucumber juice. Niacin was not appreciably altered, whereas pantothenic acid was not utilized by any of the organisms tested. In fact, most of the yeasts appeared to synthesize pantothenic acid. In two laboratory fermentations, the maximum concentrations of the three vitamins in the brine were reached in the first 5 to 6 days, and did not greatly change thereafter. They concluded that regardless of microbiological activity there seemed to be an abundant supply of these three vitamins for the growth of L. plantarum.

The same workers then investigated the role of biotin in the nutrition of L. plantarum, and Aerobacter cloacae isolated from cucumber fermentation (46). They showed that L. plantarum reduced the biotin content slightly when grown in cucumber juice, whereas it was markedly depleted when the organisms were grown in an assay medium containing biotin. They concluded that the cucumber juice probably contains one or more substances, lipoidal in nature, which are capable of substituting for biotin in supporting the growth of L. plantarum. Furthermore, A. cloacae can synthesize biotin in

an assay medium if it is present in only minute amounts. If, however, substantial amounts of biotin are initially present, a marked reduction of it occurs.

In the same year, Costilow and Fabian (11) identified four isolates, obtained from four different commercial cucumber fermentations, as L. plantarum. They studied the requirements of these isolates for biotin, niacin and pantothenic acid. Their results agreed with those of Rosen and Fabian (45). All strains of L. plantarum examined required biotin, niacin, and pantothenic acid. In another investigation (9) by the same workers on the rate of diffusion and concentrations of these vitamins during cucumber fermentation, six commercial and several laboratory fermentations were studied. In commercial salt stock fermentations, the vitamins diffused rapidly from the cucumbers after brining and reached their maximum concentrations in from 5 to 7 days, with no great changes after that time even when tested 64 days after salting. However, in all instances examined, the vitamin levels were well above those required by L. plantarum.

When the effect of some brine microorganisms, L. plantarum, a coliform, and four isolates of yeast, on the vitamin levels in brine was studied, they reported that bacteria and yeast had no effect on the niacin content (10). Only L. plantarum caused a significant reduction in pantothenic acid, but biotin levels were lowered by both bacterial cultures and

all four yeasts. In addition, they observed some synthesis of pantothenic acid by Torulaspora rosei and Hansenula subpelliculosa.

Amino Acids in Cucumber Fermentation

Extensive studies have been carried out on the amino acid requirements of L. plantarum (52). However, before 1953 no information was found available on the amino acids content of cucumbers and cucumber pickles. In 1941 Camillo et al. (5) reported that the protein content of fresh cucumbers varied from 0.7 to 1.4 per cent. When the fresh cucumbers were made into salt stock, the protein content was decreased from 1.427 per cent in fresh cucumbers to 1.038 percent in the salt stock. The nutritional data by the H. J. Heinz Company (56) indicates 1.1 per cent total protein in fresh cucumbers; 0.8 per cent for fresh cucumber pickles; and 0.7 per cent for dill pickles.

In 1953 Costilow and Fabian (11) were the first to study the amino acids content of pickle brine during fermentation. They investigated the amino acid requirements of four isolates of L. plantarum obtained from commercial fermentations and showed that leucine, isoleucine, valine, glutamic acid, tryptophane, cystine and threonine were necessary for growth. Since other studies (38, 49) have established that L. plantarum 17-5 did not require threonine in certain

media, Costilow and Fabian (11) indicated that the requirements of L. plantarum for cystine and threonine depends on the basal medium used for testing. In another study on the amino acids available for the growth of L. plantarum in brine, six commercial and six laboratory fermentations were examined (9). The amino acids reached their maximum concentrations in 10 to 19 days in the commercial fermentations, and in 15 to 20 days in the laboratory experiments. In all cases examined, a reduction of tryptophane occurred during fermentation about the same time that yeast activity was greatest. Therefore, yeasts may either utilize or destroy large quantities of this amino acid. On the other hand, coliforms and acid-forming bacteria did not contribute greatly to the destruction of tryptophane. Cystine showed a low concentration level even after 19 days of fermentation. This reduction which occurred in the commercial fermentations, disappeared as fermentation proceeded. The concentrations of leucine, isoleucine, valine and glutamic acid were affected by one or more of the brine microorganisms. However, in all instances, there was an abundant supply of them for the growth of L. plantarum. Thus, the great activity of yeasts and/or the coliform organisms, especially at the beginning of the fermentation, might result in critical concentrations of tryptophane and cystine for the growth of L. plantarum. All the other amino acids are not thought to be limiting factors. In the same

year Costilow and Fabian (10) extended their work on the effect of brine microorganisms on the amino acids content of cucumber brines. They observed that yeasts and L. plantarum reduced to varying degrees the concentrations of leucine, isoleucine, valine and tryptophane. The coliform organisms showed no effect. On the other hand, the levels of glutamic acid and cystine were greatly lowered by the coliform organisms, whereas yeast had no such effect. In fact synthesis of glutamic acid and cystine by yeasts was noted.

EXPERIMENTAL METHODS

Semicommercial Fermentations Studied and Methods of Sampling

The first experiment began August 3, 1953, at the salting station of the H. W. Madison Company, Mason, Michigan. Twelve barrels were filled with size 2 cucumbers of the variety MR-17 (about 4 1/2 bushels of cucumbers per barrel). The cucumbers used in all barrels were taken from the same field, to minimize variations as much as possible. A 30° salometer schedule (7.9 per cent NaCl) was followed in salting, i.e., the initial salt concentration was 30° salometer which was raised two degrees every week to 45°, and one degree weekly till the end of the experiment, September 13, 1953. The barrels were left outdoors.

The following additions, in terms of per cent in these and all subsequent experiments, are based on the weight of cucumbers and brine:

1953 Experiments

Barrel 1: nothing added (control).

Barrel 2: 0.5% sucrose added at the time of salting.

Barrel 3: 0.5% sucrose added on the 7th day of fermentation.

Barrel 4: 1.0% sucrose added at the time of salting.

Barrel 5: 1.0% sucrose added on the 7th day of fermentation.

Barrel 6: 1.0% sucrose divided into three equal parts: 1/3 added at the time of salting, 1/3 added on the 7th day, and 1/3 added on the 14th day.

In this way, the influence of the addition of different concentrations of sucrose to the cucumber salt stock was investigated as to its effect when added at different times.

Similarly, the influence of adding tryptophane and cystine to fermenting cucumbers was studied, and barrels 7 and 8 were treated as follows:

Barrel 7: 0.0025% tryptophane, and 0.0025% cystine added at the time of salting.

Barrel 8: the same amounts of tryptophane and cystine added on the 7th day of fermentation.

Furthermore, to investigate the effect of the combined treatment of sugar, tryptophane and cystine, a barrel was prepared as follows:

Barrel 9: 0.0025% tryptophane, 0.0025% cystine, and 1.0% sucrose, added at the time of salting.

The effect of adding other materials, besides sugar and amino acids to cucumber salt stock, was also studied. The material chosen in this work was corn steep liquor. Accordingly, the following barrels were prepared:

Barrel 10: one ounce of corn steep liquor[1] for every gallon of brine added at the time of salting.

Barrel 11: the same amount of corn steep liquor added on the 7th day of fermentation to study its influence when added after active fermentation had started.

Barrel 12: corn steep liquor in the same concentration as in barrels 10 and 11, and also 1.0% sucrose added as follows: 1/3 added at the time of salting, 1/3 on the 7th day, and 1/3 on the 14th day of fermentation. This barrel was included to study the effect of the combined treatment of corn steep liquor and sucrose.

The corn steep liquor used in these experiments had the following composition:

Substance	Per cent	Substance	Percent
Dry substance	54.5 - 57.5	SO_2 (avg.)	0.05
Reducing sugars (max.)	5.0	protein (total)	45 - 50
Acid as lactic (min.)	20.0		
	pH (max.)	4.2	

A stainless steel tube, 30 inches long and 3/16 inches inside diameter and sealed at one end with holes spaced 9.5

[1]Corn steep liquor obtained from Corn Products Refinery Co., Argo, Illinois.

inches apart starting at the sealed end, was used in sampling. Samples were taken through a hole in the head of each barrel by inserting the tube and then syphoning the brine into sterile sample tubes. In this way it was felt that a composite and representative sample was obtained.

During the first week samples were taken every day to follow the fermentation closely. During the second week samples were taken every two days except barrels 1, 8, 11 and 12, from which the samples were taken on four successive days after the specific treatment. During the 3rd week sampling was made every three days except the control barrel and barrels 6 and 12 where sugar or corn steep liquor was added on the 14th day. In these barrels samples were taken for four consecutive days after the specific treatment. During the 4th, 5th, and 6th weeks, samples were taken every four days.

On removal of the samples from the barrels, they were immediately transferred to the laboratory where chemical and bacteriological analyses were made, after which they were frozen at -18.5° C until the microbiological assays were carried out.

Chemical and Bacteriological Analyses

(1) The brine samples were titrated with standard NaOH solution to determine the titratable acidity which was expressed as grams lactic acid per 100 ml of brine.

(2) The salt concentration was determined by titration with standard $AgNO_3$ using dichlorofluroescein as indicator (35).

(3) Total sugars in the brine samples were determined by the Munson and Walker Method (39). The results were expressed as grams invert sugar per 100 ml of brine.

(4) The pH of the brine was determined using a CENCO glass electrode titration-pH meter.

(5) Total bacteria count was made using dextrose tryptone agar.

(6) Total yeast count was made on dextrose agar acidified with 5.0 ml of five per cent tartaric acid per 100 ml of the medium.

(7) Acid-forming bacteria count was made using V-8 medium (23).

(8) Total coliform count was made using single strength lauryl tryptose broth inoculated in triplicate with various dilutions of the brine samples.

All plates and tubes were incubated at 30°C for all microbial analyses. Three days incubation was allowed for the total bacteria count and for the acid formers, two days for the coliforms, and seven days for the yeast.

1954 Experiments

A second set of experiments was started on July 30, 1954, under the same conditions and at the same place as the first one. This time, only six barrels were put down.

The cucumbers used were of size 2 of the variety SR-6. The same salting schedule as in the 1953 experiments was followed. The cucumbers and brine were treated in the following manner:

Barrels 1 and 2: no treatment (control).

Barrels 3 and 4: 0.5 per cent sucrose added to each barrel on the 7th day of fermentation.

Barrels 5 and 6: corn steep liquor, one ounce per gallon of brine, and one per cent sucrose divided into three equal parts: 1/3 added at salting, 1/3 on the 7th day, and 1/3 on the 14th day of fermentation for each barrel.

The above experiments were carried out as checks on the 1953 experiments which showed a slight increase in the lactic acid produced in the barrels with the above mentioned treatments over the control barrel. Thus, it was felt necessary to investigate again the influence of the specific treatments on lactic acid formation.

The chemical and bacteriological analyses were carried out, using the same methods as in the former experiments, except that determinations of reducing sugars and the microbiological assays were not made.

Laboratory Fermentations Studied

A third set of experiments was carried out in the laboratory with the object of investigating the effect of the addition of sugar, amino acids and corn steep liquor on the salt

stock under ideal conditions. Eighteen flasks were prepared in the following manner:

The cucumbers were washed thoroughly and the juice was extracted. Hundred-ml portions of the cucumber juice were then placed in 500-ml Erlenmeyer flasks. To each flask 100 ml of one per cent NaCl was added. This made the final concentration of NaCl in the flasks 0.5 per cent. Sucrose, amino acids, or corn steep liquor, according to the specific treatment, were added to the flasks. The flasks were sterilized at 15 lbs. for 15 minutes. A pure culture of L. plantarum 17-5 was first activated by inoculation into microinoculum broth, then centrifuged and the bacterial cells were suspended in physiological salt solution. Each flask was then inoculated with 0.5 ml of the bacterial saline suspension. All flasks were incubated at 30° C. According to Bergey's Manual of Determinative Bacteriology (3), these conditions are considered to be the optimum for the growth of L. plantarum. Samples were taken periodically from the flasks for determination of percentage acid formed, and the lactic acid bacteria count. The period of the experiment was 25 days. The specific treatments were:

4 flasks (1, 2, 3, 4)	:	no treatment (control).
3 flasks (5, 6, 7)	:	0.5 percent sucrose added.
3 flasks (8, 9, 10)	:	1.0 per cent sucrose added.
2 flasks (11, 12)	:	0.0025 per cent tryptophane and 0.0025 per cent cystine added.

2 flasks (13, 14) : 0.0025 per cent tryptophane, 0.0025 per cent cystine and 1.0 per cent sucrose added.

2 flasks (15, 16) : 0.821 per cent corn steep liquor added.

2 flasks (17, 18) : 0.821 per cent corn steep liquor and 1.0 per cent sucrose added.

It should be noted that the concentration of corn steep liquor used was the same as that for the barrel experiments.

A fourth set of experiments, designed the same as the third one, was carried out in the laboratory to study the relation between the reducing sugars, the amount of acid formed and the lactic acid bacteria count. The following additions were made:

2 flasks (1, 2) : no treatment (control).

2 flasks (3, 4) : 0.5 per cent sucrose was added.

2 flasks (5, 6) : 1.0 per cent sucrose was added.

2 flasks (7, 8) : 0.0025 per cent tryptophane and 0.0025 per cent cystine added.

2 flasks (9, 10): 0.0025 per cent tryptophane, 0.0025 per cent cystine and 1.0 per cent sucrose added.

2 flasks (11, 12): 0.821 per cent corn steep liquor added.

2 flasks (13, 14): 0.821 per cent corn steep liquor and 1.0 per cent sucrose added.

Determinations of the amount of acid formed and the lactic acid bacteria count were carried out periodically following the same methods as previously described. The reducing sugars were determined by the Munson and Walker Method (39), at the start and at the end of the experiment. The experiment lasted 35 days.

Microbiological Assays for Vitamins and Amino Acids

In the first set of experiments made in 1953, it was of interest to study the effect of the different materials added to the cucumber brines on the amino acids and vitamins available for the growth of Lactobacillus plantarum. Consequently, treatment of the brine samples to release the vitamins, or to free the amino acids from the protein material prior to running the microbiological assays, was unnecessary.

The brine samples were removed from the freezer and allowed to thaw at 4.5°C just prior to running the assay. They were then stored in the refrigerator under toluene.

At the time of assaying, proper dilutions were made from the original brine samples as needed. The methods employed in the assays were essentially the same as those described in "Methods of Vitamin Assays" (51). The assays for valine and glutamic acid were carried out following the method described by Sauberlich and Baumann (47), using medium I and L. plantarum 17-5 as the test organism. The dehydrated

media prepared by Difco Laboratories, Inc., were used to assay the samples for biotin, pantothenic acid, niacin, leucine, isoleucine, tryptophane and cystine. L. plantarum 17-5 was the test organism used for the assays of biotin, niacin, pantothenic acid and tryptophane. Leuconostoc mesenteroides (strain P-60) was used for cystine, leucine and isoleucine assays.

RESULTS

I - Influence of Added Sugar on Cucumber Fermentations

It is well known that two identical cucumber fermentations might show some variations in the amount of lactic acid formed as well as in many other aspects of the fermentation. Before presenting the effects of the sugar treatments on lactic acid formation, it is more appropriate to study the variations normally present in brines of similar lots. Table 3 shows that the highest value of variation in brine acidity between individual fermentations, which received similar treatments in the 1954 experiment, is 0.10 per cent. Greater variations in brine acidity, however, might appear if comparisons are made between similar treatments prepared in 1953 and 1954. It is noted that three control fermentations were prepared in this investigation. However, other cucumber salt stocks did not receive any treatment until the 7th day of fermentation, e.g., barrels 3, 5, 8 and 11 in 1953; and barrels 3 and 4 in 1954. Therefore, lactic acid formation in brines of these barrels could be considered similar to that of the control fermentations only during the first week after brining. When comparison is made between these barrels and the control barrels, the greatest difference observed was 0.10 per cent (Table 5). Therefore, it is logical to assume

that, under the conditions of these experiments, if a specific treatment resulted in an increase greater than 0.10 per cent lactic acid the difference should be considered significant.

Effect of 0.5 per cent sucrose on lactic acid formation. Table 1 indicates that, in the 1953 experiments, the amount of lactic acid formed in the brine in barrels 1, 2 and 3 generally increased after salting, reached a maximum in from 10 to 21 days, with little change thereafter. In individual experiments it seemed as if the addition of sugar might have resulted in a slight increase in acidity as compared with the case where no sugar was added. For example, in barrels 2 and 3, brine acidity reached a level of 0.6 per cent on the 11th day. The same level of acidity in the control lot was attained after 18 days of fermentation. Yet, this apparent rise in acidity over the control fermentation did not exceed 0.10 per cent. Consequently, it could not be attributed to the specific treatment. On the other hand, it was noted that the amount of lactic acid formed after 13 days in barrel 3, was 0.67 per cent as compared to 0.52 per cent in the control. Also, brine acidity reached a level of 0.72 per cent after three weeks and remained slightly higher than the control for the rest of the examination period. Thus, it was deemed of interest to repeat the experiment in which sugar was added after active fermentation had started, and to check

TABLE 1

EFFECT OF DIFFERENT TREATMENTS ON LACTIC ACID FORMATION AND pH MEASUREMENTS IN BRINES OF SEMICOMMERCIAL CUCUMBER SALT STOCK

1953 Fermentations

Time After Brining	Barrels filled with size 2 cucumbers of the variety MR-17											
	1		2		3		4		5		6	
	A	pH	A	pH	A	pH	A	pH	A	pH	A	pH
0-3 hrs.	*	7.6	*	7.8	*	7.9	*	7.8	*	7.9	*	7.9
1 day	0.01	7.2	0.01	7.2	0.01	7.3	0.01	7.1	0.01	7.2	0.01	7.4
2 days	0.15	5.9	0.07	6.3	0.07	6.7	0.07	6.1	0.07	6.1	0.07	6.4
3	0.30	5.0	0.30	4.7	0.30	5.8	0.30	4.8	0.21	4.9	0.22	4.7
4	0.30	4.6	0.30	4.5	0.30	4.5	-	-	0.25	4.6	0.30	4.5
5	-	-	0.27	4.5	-	-	0.34	4.5	0.33	4.5	0.45	4.5
6	0.37	4.5	0.37	4.5	0.45	4.5	0.37	4.5	0.35	4.6	0.42	4.5
7	0.37	4.5	0.37	4.4	0.45	4.4	0.37	4.5	0.35	4.6	-	-
8	-	-	-	-	0.48	4.4	-	-	0.45	4.5	0.37	4.3
9	0.45	4.5	0.45	4.3	0.52	4.3	0.45	4.4	0.52	4.5	0.45	4.3
10	-	-	-	-	0.52	4.3	-	-	0.45	4.5	0.52	4.3
11	0.52	4.5	0.60	4.3	0.60	4.3	0.52	4.4	0.45	4.5	0.60	4.3
13	0.52	4.5	0.60	4.3	0.67	4.3	0.52	4.3	0.45	4.5	0.60	4.3
15	0.52	4.4	0.60	4.2	0.60	4.3	0.60	4.3	0.52	4.3	0.67	4.2
16	-	-	-	-	-	-	-	-	-	-	0.67	4.2
17	-	-	-	-	-	-	-	-	-	-	0.67	4.2
18	0.60	4.1	0.67	4.2	0.67	4.2	0.73	4.2	0.60	4.2	0.67	4.2
21	0.60	4.1	0.67	4.2	0.72	4.2	0.60	4.2	0.60	4.3	0.60	4.2
25	0.60	4.1	0.60	4.0	0.67	4.1	0.60	4.1	0.60	4.1	0.60	4.2
29	-	-	0.67	4.1	0.70	4.1	0.62	4.1	0.52	4.1	0.67	4.0
33	0.60	4.2	0.60	4.1	0.72	4.1	0.60	4.1	0.60	4.1	0.60	4.1
37	0.60	4.1	0.60	4.1	0.72	4.1	0.60	4.1	0.60	4.2	0.60	4.1
41	0.60	4.1	0.67	4.1	0.73	4.1	0.67	4.1	0.60	4.2	0.60	4.1

- = no determination made
* = less than 0.01 per cent lactic acid
A = per cent lactic acid

Barrel 1 = Control, nothing added.
Barrel 2 = 0.5% sucrose, added at the time of salting.
Barrel 3 = 0.5% sucrose, added on the 7th day of fermentation.
Barrel 4 = 1.0% sucrose, added at the time of salting.
Barrel 5 = 1.0% sucrose, added on the 7th day of fermentation.
Barrel 6 = 1.0% sucrose, equally divided into 3 portions; 1/3 added at the time of salting, 1/3 on the 7th day, and 1/3 on the 14th day of fermentation.

TABLE 1 - Continued

Time After Brining	Barrels filled with size 2 cucumbers of the variety MR-17											
	7		8		9		10		11		12	
	A	pH	A	pH	A	pH	A	pH	A	pH	A	pH
0-3 hrs.	*	7.8	*	7.9	*	7.9	*	6.2	*	7.8	*	7.4
1 day	*	7.3	*	7.2	*	7.0	*	6.5	*	7.1	*	6.8
2 days	0.07	6.5	0.05	6.3	0.07	6.3	0.15	6.2	0.07	6.0	0.07	6.2
3	0.15	5.3	0.22	4.7	0.30	4.7	0.22	4.9	0.22	4.7	0.30	4.8
4	0.30	4.6	0.30	4.5	-	-	0.37	4.5	0.33	4.5	0.34	4.5
5	0.30	4.5	0.30	4.4	0.37	4.5	0.48	4.4	0.34	4.5	0.30	4.5
6	0.37	4.5	0.45	4.5	0.45	4.4	0.52	4.5	0.37	4.5	0.45	4.5
7	0.40	4.5	0.42	4.3	0.45	4.4	0.45	4.4	0.37	4.5	0.45	4.3
8	-	-	0.45	4.3	-	-	-	-	0.52	4.4	0.52	4.4
9	0.37	4.5	0.45	4.4	0.52	4.3	0.60	4.3	0.45	4.3	0.52	4.4
10	-	-	0.45	4.4	-	-	-	-	0.52	4.4	0.60	4.4
11	0.45	4.4	0.45	4.5	0.52	4.3	0.60	4.4	0.60	4.4	0.60	4.4
13	0.52	4.3	-	4.3	0.52	4.3	0.67	4.4	0.60	4.3	0.60	4.3
15	0.60	4.2	0.60	4.3	0.62	4.2	-	-	0.67	4.3	0.75	4.3
16	-	-	-	-	-	-	-	-	-	-	0.75	4.3
17	-	-	-	-	-	-	-	-	-	-	0.75	4.3
18	0.75	4.2	0.60	4.2	0.65	4.2	0.75	4.3	0.67	4.3	0.75	4.3
21	0.67	4.2	0.52	4.2	0.75	4.2	0.67	4.3	0.75	4.2	0.67	4.2
25	0.60	4.1	0.60	4.1	0.67	4.1	0.75	4.2	0.67	4.1	0.75	4.2
29	0.60	4.1	0.60	4.1	0.67	4.1	0.75	4.3	0.75	4.1	0.75	4.2
33	0.60	4.1	0.60	4.2	0.60	4.1	0.67	4.2	0.67	4.1	0.75	4.1
37	0.60	4.1	0.52	4.2	0.67	4.1	0.75	4.2	0.67	4.1	0.71	4.1
41	0.60	4.1	0.52	4.1	0.65	4.1	0.67	4.2	0.75	4.1	0.75	4.1

Barrel 7 = 0.0025% cystine, and 0.0025% tryptophane, added at the time of salting.
Barrel 8 = 0.0025% cystine, and 0.0025% tryptophane, added on the 7th day of fermentation.
Barrel 9 = 0.0025% cystine, 0.0025% tryptophane, and 1.0% sucrose, added at the time of salting.
Barrel 10= corn steep liquor (1 oz/gallon of brine), added at the time of salting.
Barrel 11= corn steep liquor (1 oz/gallon of brine), added on the 7th day of fermentation.
Barrel 12= corn steep liquor (1 oz/gallon of brine), and 1.0% sucrose was equally divided into three portions; 1/3 added at the time of salting, 1/3 on the 7th day, and 1/3 on the 14th day of fermentation.

TABLE 2

PER CENT SALT IN BRINES OF SEMICOMMERCIAL CUCUMBER SALT STOCK

1953 Fermentations

Time After Brining	No. of Barrel											
	1	2	3	4	5	6	7	8	9	10	11	12
0-3 hrs.	13.5	13.8	14.0	13.1	13.0	12.9	13.2	13.9	13.3	13.2	12.5	14.2
1 day	9.6	9.1	9.5	8.9	9.6	8.8	9.4	9.5	8.7	8.7	8.7	8.4
2 days	7.9	8.1	8.1	8.5	8.7	8.5	8.3	8.6	8.1	7.9	7.8	8.6
3	7.4	7.7	7.3	7.5	7.4	7.3	7.3	7.3	7.1	7.1	7.3	7.5
4	7.5	7.9	7.3	7.4	7.1	7.4	7.2	7.5	7.6	7.3	7.2	7.5
5	7.2	7.3	7.3	7.6	7.6	7.1	7.1	7.7	7.7	7.4	7.3	7.4
6	7.7	7.6	7.2	7.6	7.6	7.3	7.3	7.7	7.4	7.1	7.1	7.2
7	7.1	7.9	7.3	7.5	7.5	7.6	7.8	7.5	7.5	7.8	7.4	7.4
8	-	-	8.5	-	8.3	8.3	-	8.8	-	-	8.0	7.9
9	8.8	8.6	8.4	8.4	8.5	8.3	8.4	9.0	8.0	8.3	8.3	8.5
10	-	-	8.7	-	8.2	8.5	-	9.1	-	-	8.3	8.6
11	8.9	9.1	8.7	8.8	9.0	8.6	8.8	9.2	8.3	8.7	8.7	8.6
13	9.1	9.2	8.8	9.0	8.5	8.6	8.4	9.2	8.1	8.9	8.3	8.9
15	9.5	9.7	9.6	8.9	9.0	8.4	8.8	9.6	8.6	8.8	8.3	8.8
16	-	-	-	-	-	9.9	-	-	-	-	-	9.2
17	-	-	-	-	-	9.5	-	-	-	-	-	9.5
18	9.6	9.9	9.4	9.4	9.2	9.6	9.8	9.6	9.3	9.2	9.5	9.4
21	9.6	10.1	9.6	10.0	9.6	10.0	10.0	9.5	9.2	9.6	9.9	9.4
25	9.8	10.1	9.9	10.0	10.1	10.1	10.3	9.8	9.9	10.1	10.2	10.5
29	10.4	10.6	10.0	10.1	10.3	10.3	10.5	10.1	10.2	10.0	10.4	10.2
33	10.8	10.9	10.6	10.6	10.6	10.7	11.2	10.4	10.7	10.9	11.1	10.7
37	10.8	10.9	10.6	10.6	10.7	10.6	11.1	10.7	11.0	10.8	11.2	10.8
41	10.7	10.9	10.6	11.0	10.8	10.7	11.1	10.8	11.5	10.9	11.7	10.9

TABLE 3

EFFECT OF SUGAR AND CORN STEEP LIQUOR TREATMENTS ON LACTIC ACID FORMATION AND pH MEASUREMENTS IN BRINES OF SEMICOMMERCIAL CUCUMBER SALT STOCK

1954 Fermentations

Time After Brining	Barrels filled with size 2 cucumbers of the variety SR-6												
	1		2				3		4				
	A	pH	A	pH	M_1	d_{1-2}	A	pH	A	pH	M_2	d_{3-4}	$D_{M_1-M_2}$
0-3 hrs.	*	7.7	*	7.7	*	-	*	7.6	*	7.9	*	-	-
2 days	0.05	5.4	0.05	5.6	0.05	0	0.06	5.5	0.05	5.4	0.05	0.01	0
3	0.24	4.4	0.22	4.4	0.23	0.02	0.21	4.5	0.31	4.4	0.26	0.10	0.03
4	0.34	4.1	0.28	4.2	0.31	0.06	0.30	4.1	0.35	4.1	0.32	0.05	0.01
5	0.40	4.1	0.30	4.4	0.35	0.10	0.37	4.3	0.40	4.1	0.38	0.03	0.03
7	0.44	4.0	0.39	4.0	0.41	0.05	0.44	4.0	0.45	4.0	0.44	0.01	0.03
8	-	-	-	-	-	-	0.56	4.0	0.53	4.0	0.54	0.03	-
9	0.45	4.1	0.52	4.0	0.48	0.07	0.57	3.9	0.58	4.1	0.57	0.01	0.09
12	0.62	4.0	0.56	4.0	0.59	0.06	0.61	4.0	0.64	4.0	0.62	0.03	0.03
14	0.56	4.0	0.50	3.9	0.53	0.06	0.58	3.9	0.58	4.0	0.58	0	0.05
18	0.61	3.9	0.58	3.8	0.60	0.03	0.61	3.8	0.60	3.9	0.60	0.01	0
21	0.62	3.9	0.69	3.9	0.65	0.07	0.62	3.9	0.65	3.9	0.63	0.03	0.02
25	0.63	3.9	0.62	3.8	0.62	0.01	0.64	3.8	0.57	3.9	0.60	0.07	0.02
29	0.88	3.9	0.51	3.8	0.54	0.07	0.61	3.8	0.64	3.8	0.62	0.03	0.08
33	0.59	4.0	0.58	3.9	0.58	0.01	0.68	3.9	0.64	4.0	0.66	0.04	0.08
37	0.54	3.8	-	-	-	-	0.66	3.8	0.64	3.8	0.65	0.02	-
41	0.55	3.8	0.64	3.8	0.59	0.09	0.63	3.8	0.64	3.9	0.63	0.01	0.04

- = no determination made.
* = less than 0.01% lactic acid.
A = per cent lactic acid.
M = average acidity values.
d = difference in acidity between specific treatment and the control.
$D_{M_1-M_2}$ = difference in acidity between M_1 and M_2.

Barrels 1, 2 = control, nothing added.
Barrels 3, 4 = 0.5% sucrose added on the 7th day of fermentation.

TABLE 3 - Continued

Time After Brining	Barrels filled with size 2 cucumbers of the variety SR-6						
	5		6				
	A	pH	A	pH	M_3	d_{5-6}	$D_{M_1-M_3}$
0-3 hrs.	*	7.6	*	7.5	*	-	-
2 days	0.07	5.4	0.09	5.1	0.08	0.02	0.03
3	0.22	4.5	0.27	4.3	0.24	0.05	0.01
4	0.33	4.1	0.31	4.0	0.32	0.02	0.01
5	0.35	4.2	0.31	4.1	0.33	0.04	0.02
7	0.43	4.0	0.40	4.0	0.41	0.03	0
9	0.59	4.0	0.60	3.9	0.59	0.01	0.11
12	0.62	4.0	0.61	4.0	0.61	0.01	0.02
14	0.58	3.9	0.59	3.9	0.58	0.01	0.05
16	0.71	4.0	0.75	3.9	0.73	0.04	-
18	0.75	3.9	0.70	3.8	0.72	0.05	0.12
21	0.74	3.9	0.72	3.9	0.73	0.02	0.12
25	0.75	3.8	0.76	3.8	0.75	0.01	0.13
29	0.73	3.8	0.68	3.8	0.70	0.05	0.16
33	0.69	3.9	0.71	3.9	0.70	0.02	0.12
37	0.70	3.8	0.70	3.8	0.70	0	-
41	0.70	3.8	0.70	3.8	0.70	0	0.11

$D_{M_1-M_3}$ = difference in acidity between M_1 and M_3.

Barrels 5, 6 = corn steep liquor (1 oz/gallon of brine), and 1.0% sucrose was equally divided into 3 portions; 1/3 added at the time of salting, 1/3 on the 7th day, and 1/3 on the 14th day of fermentation.

TABLE 4

PER CENT SALT IN BRINES OF SEMICOMMERCIAL CUCUMBER SALT STOCK

1954 Fermentations

Time After Brining	No. of Barrel					
	1	2	3	4	5	6
0-3 hrs.	13.0	12.8	12.8	13.3	13.5	13.3
2 days	8.3	7.7	7.7	7.2	8.4	9.2
3	7.4	7.5	7.5	7.1	7.9	8.4
4	7.5	7.4	7.4	7.5	7.4	8.4
5	7.5	7.2	7.2	7.5	7.5	7.6
7	7.7	7.4	7.4	7.7	7.7	7.8
9	8.1	8.2	8.2	8.0	8.4	8.1
12	8.4	8.7	8.7	8.6	8.8	8.4
14	8.8	8.3	8.3	8.4	8.7	8.8
16	-	-	-	-	9.1	9.0
18	9.5	9.3	9.3	9.1	9.1	8.9
21	9.8	9.0	9.0	9.7	9.8	9.5
25	9.4	9.6	9.6	9.9	9.8	9.5
29	9.5	9.8	9.8	9.5	9.5	9.7
33	9.8	9.5	9.5	9.8	9.6	9.8
37	10.8	9.9	9.6	9.9	9.8	9.6
41	10.8	10.5	10.4	10.2	10.9	10.5

TABLE 5

DIFFERENCE IN BRINE ACIDITY DURING THE FIRST WEEK BETWEEN SIMILAR LOTS OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS PREPARED IN TWO DIFFERENT SEASONS

1953 and 1954 Fermentations

Time After Brining	1953 Fermentations No. of Barrel						1954 Fermentations No. of Barrel					
	1 A	3 A	5 A	8 A	11 A	D_1	1 A	2 A	3 A	4 A	D_2	D_3
2 days	0.15	0.07	0.07	0.05	0.07	0.10	0.05	0.05	0.06	0.05	0.01	0.10
3	0.30	0.30	0.21	0.22	0.22	0.09	0.24	0.22	0.21	0.31	0.10	0.10
4	0.30	0.30	0.25	0.30	0.33	0.08	0.34	0.28	0.30	0.35	0.07	0.10
5	-	-	0.33	0.30	0.34	0.04	0.40	0.30	0.37	0.40	0.10	0.10
6	0.37	0.45	0.35	0.45	0.37	0.10	-	-	-	-	-	-
7*	0.37	0.45	0.35	0.42	0.37	0.10	0.44	0.39	0.44	0.45	0.06	0.10

- = no determinations made.
* = determinations made before each barrel received its specific treatment.
A = per cent lactic acid.

D_1 = maximum difference in acidity between barrels 1, 3, 5, 8 and 11 in 1953.

D_2 = maximum difference in acidity between barrels 1, 2, 3 and 4 in 1954.

D_3 = maximum difference in acidity between the 1953, and the 1954 fermentations.

again the influence of the treatment. Consequently in 1954, control barrels 1 and 2 and two fermentations where sugar was added on the 7th day after brining (barrels 3 and 4) were prepared. The results of this experiment are included in Table 3. In general, brine acidity increased after salting and reached a maximum of about 0.6 per cent approximately on the 16th day of fermentation. Thereafter, small changes occurred. These results are in close agreement with the general trend of acidity changes obtained before, i.e., in the 1953 experiment. Also, the average brine acidity of the sugar treatments did not significantly increase over that of the no-sugar treatments. Results in Table 3 show that the maximum difference in acidity, i.e., $D_{M_1-M_2}$, between both treatments was 0.09 per cent on the 9th day. This value is still within the limit of variations that occurred between individual barrels of similar treatments.

Therefore, addition of 0.5 per cent sucrose at the time of salting, or on the 7th day of fermentation had no effect in inducing faster or greater acid formation than in the case where no sugar was added.

Furthermore, to clarify this matter, a laboratory experiment was carried out in which very favorable conditions were created for the growth of L. plantarum. The complete results of this experiment are given in Table 6. It is evident from these results that the average brine acidity values of both the sugar and no-sugar treatments were almost identical

throughout the examination period. The highest increase of acidity over the control, i.e., $D_{M_1-M_2}$, was only 0.02 per cent.

The flask experiment was repeated again in the same manner, except that two flasks were used for each treatment and that the reducing sugars of the brine were determined at the start and at the end of the experiment, i.e., after 35 days. Results in Table 7 confirm the previous findings. Therefore, addition of 0.5 per cent sucrose at the time of salting did not result in more acid formation even under very favorable conditions. It should be noted that the total titratable acidity values were somewhat lower in the 4th experiment than those observed in the 3rd experiment. This might be attributed to the fact that different varieties of cucumbers were used in each experiment since the cucumbers were supplied by local distributors. The reducing sugar contents expressed as per cent invert sugar, are recorded in Table 8. As a result of added sugar, the invert sugar values were found to be higher in flasks 3 and 4 at the start and at the end of the experiment than the corresponding values obtained in the control flasks. However, the amounts of reducing sugars utilized by the organisms after 35 days were almost the same in all four flasks. This would indicate that the added sugar was not converted into acid. It also indicates that L. plantarum can only use a certain amount of sugar and produces a certain amount of acid irrespective of the amount of sugar present.

Under normal conditions, the cucumbers probably have sufficient sugar present to meet the needs of L. plantarum.

Effect of 1.0 per cent sucrose on lactic acid formation. Table 1 shows that in barrels 4 and 5, no significant increase in acidity occurred as a result of adding sucrose. At any given time the slight increase in brine acidity over the control never exceeded 0.10 per cent. Only in barrel 4 was there an increase of 0.13 per cent acid on the 18th day. This difference did not persist for any considerable time and therefore, is considered insignificant. Also, addition of sugar on the 7th day of fermentation did not cause an appreciable increase in acidity on the following days. It is true that the percentage lactic acid slightly increased from 0.35 per cent on the 7th day to a level of 0.52 per cent on the 9th day after the sugar treatment. But the total titratable acidity decreased to 0.45 per cent on the 10th day and showed the same value on the 13th day. These fluctuations in acidity are not significant inasmuch as the control fermentation showed a similar increase during the same period of time. They are probably due to experimental error and/or normal variations between samples and microflora.

Inasmuch as no appreciable difference in brine acidity was observed to be induced by the addition of 1.0 per cent sucrose either at the start or after seven days, it seemed desirable to approach the study in another manner. It was

thought that, by adding 1.0 per cent sucrose in small quantities at intervals over an extended period of time, the added sugar might be converted more efficiently into acid, thereby resulting in the formation of an increased quantity of lactic acid.

Accordingly, barrel 6 was prepared and 1.0 per cent sucrose of the weight of cucumbers and brine was divided into three equal portions, 1/3 added at the time of salting, 1/3 on the 7th day, and 1/3 on the 14th day. When the first portion of sugar was added, the amount of lactic acid formed after 6 days was 0.42 per cent as compared to 0.37 per cent in the control. When the second portion of sugar was added, the brine acidity was 0.6 per cent on the 13th day as compared to 0.52 per cent in the control. Therefore, the increase in acidity was 0.05 per cent as a result of the first addition of sugar and 0.08 per cent as a result of the second addition. These differences are insignificant as greater differences might occur between duplicate treatments within one lot. Similarly, addition of the third portion of sugar on the 14th day did not result in an appreciable rise in acid production. Therefore, the addition of 1.0 per cent sucrose in small quantities at seven-day intervals throughout the fermentation showed no significant effect on the amount of lactic acid formed.

When the fermentations were carried out in flasks under optimum conditions, similar results were obtained (Fig. 4,

part A). The average acidity values of the sugar and no-sugar treatments were almost the same (Tables 6 and 7). Determination of the reducing sugars at the start and at the end of the experiment (Table 8) indicates that sugar utilization by L. plantarum was not enhanced as a result of the treatment. On the contrary, the amounts of reducing sugars utilized in flasks 5 and 6 were even slightly lower than those of the control flasks. These small differences however, could be considered in the range of experimental error.

Since no significant increase in brine acidity occurred as a result of addition of sugar, in neither the 1953 nor the 1954 experiments, the average acidity values of all sugar treatments are graphically presented in Fig. 1.

A series of observations was also made on the changes occurring in contents of the reducing sugars of brines of the 1953 fermentations (Table 9). In Fig. 2, part B, the curve representing the control fermentation indicates a fairly rapid increase in reducing sugar after salting. This was mostly due to leaching of the naturally occurring sugars from the cucumbers into the brine. The reducing sugars reached a maximum about the 7th day, then decreased rapidly and could not be detected on the 25th day of fermentation. This decrease is most likely caused by the utilization of sugar by the brine microorganisms during fermentation. The curves representing the contents of reducing sugars of brines, to which 0.5 per cent sucrose was added either at the time of

salting or on the 7th day, are somewhat different. When sugar was added at the time of salting, the concentration of the reducing sugars at the beginning of the fermentation was 1.25 per cent, whereas no sugar could be detected in the control barrel. As fermentation progressed, the reducing sugars increased at first, and, like the control, reached a peak about the 6th day. However, greater amounts of reducing sugars were present, i.e., 1.697 per cent as compared to 1.008 per cent for the control. A rapid decline then occurred until the concentration was almost the same as that of the control, i.e., 0.386 per cent for barrel 2 and 0.336 per cent for the control on the 13th day. In barrel 3, the concentrations of reducing sugar were similar to that of the control during the first week of fermentation. Since 0.5 per cent sucrose was added on the 7th day, the content of reducing sugars continued to increase and reached a peak about the 9th day, then declined sharply to a value of 0.469 per cent on the 13th day. Therefore, in both barrels 2 and 3 greater amounts of reducing sugars were utilized by the brine microorganisms than in the control fermentation. Yet, Table 1 shows that this added sugar was not converted into acid since the titratable acidity did not appreciably increase.

When the amount of added sugar was increased to 1.0 per cent, similar observations were made (Fig. 2, part A). Apparently, then, sugar was not utilized mainly by the acid-forming bacteria.

TABLE 6

EFFECT OF DIFFERENT TREATMENTS ON LACTIC ACID FORMATION IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Third Flask Experiment

Time After Brining	Number of Flask														
	1 A	2 A	3 A	4 A	M_1	5 A	6 A	7 A	M_2	$D_{M_1-M_2}$	8 A	9 A	10 A	M_3	$D_{M_1-M_3}$
0-3 hrs.	*	*	*	-	-	*	*	*	-	-	*	*	*	-	-
2 days	0.70	0.65	0.70	0.70	0.68	0.67	0.68	0.70	0.68	0	0.67	0.69	0.68	0.68	0
6	0.97	0.95	0.98	0.94	0.96	0.96	0.95	0.95	0.95	0.01	0.93	0.97	0.90	0.93	0.03
11	1.10	1.08	1.07	1.06	1.07	1.09	1.06	1.04	1.06	0.01	1.05	1.05	1.06	1.05	0.02
17	1.12	1.08	-	1.07	1.09	1.11	1.13	1.09	1.11	0.02	1.10	1.04	1.12	1.08	0.01
25	1.10	1.10	-	1.10	1.10	1.10	1.10	1.10	1.10	0	1.10	1.10	1.08	1.09	0.01

- = no determination made.
* = less than 0.01% lactic acid.
A = per cent lactic acid.
M_1 = average acidity values of flasks 1, 2, 3, 4.

M_2 = average acidity values of flasks 5, 6, 7.

M_3 = average acidity values of flasks 8, 9, 10.

$D_{M_1-M_2}$ = difference in acidity between M_1 and M_2.

$D_{M_1-M_3}$ = difference in acidity between M_1 and M_3.

Flasks 1, 2, 3, 4 = control, nothing added.
Flasks 5, 6, 7 = 0.5% sucrose, added.
Flasks 8, 9, 10 = 1.0% sucrose, added.

TABLE 6 - Continued

Time After Brining	Number of Flask																
	11 A	12 A	M_4	$D_{M_1-M_4}$	13 A	14 A	M_5	$D_{M_1-M_5}$	15 A	16 A	M_6	$D_{M_1-M_6}$	17 A	18 A	M_7	$D_{M_1-M_7}$
0-3 hrs.	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-
2 days	0.66	0.67	0.66	0.02	0.64	0.68	0.66	0.02	0.72	0.71	0.71	0.03	0.73	0.73	0.73	0.05
6	0.95	0.93	0.94	0.02	0.92	0.96	0.94	0.02	1.00	1.01	1.00	0.04	1.00	1.01	1.00	0.04
11	1.07	1.05	1.06	0.01	1.05	1.02	1.03	0.04	1.12	1.20	1.16	0.09	1.18	1.17	1.17	0.10
17	1.10	1.10	1.10	0.01	1.03	1.07	1.05	0.04	1.20	1.21	1.20	0.11	1.28	1.20	1.24	0.15
25	1.10	1.08	1.09	0.01	1.04	1.08	1.06	0.04	1.20	1.24	1.22	0.12	1.29	1.29	1.29	0.19

M_4 = average acidity values of flasks 11, 12.

M_5 = average acidity values of flasks 13, 14.

M_6 = average acidity values of flasks 15, 16.

M_7 = average acidity values of flasks 17, 18.

$D_{M_1-M_4}$ = difference in acidity between M_1 and M_4.

$D_{M_1-M_5}$ = difference in acidity between M_1 and M_5.

$D_{M_1-M_6}$ = difference in acidity between M_1 and M_6.

$D_{M_1-M_7}$ = difference in acidity between M_1 and M_7.

Flasks 11, 12 = 0.0025% cystine, 0.0025% tryptophane, added.
Flasks 13, 14 = 0.0025% cystine, 0.0025% tryptophane, 1.0% sucrose, added.
Flasks 15, 16 = 0.821% corn steep liquor, added.
Flasks 17, 18 = 0.821% corn steep liquor, 1.0% sucrose, added.

TABLE 7

EFFECT OF DIFFERENT TREATMENTS ON LACTIC ACID FORMATION IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Fourth Flask Experiment

Time After Brining	No. of Flask										
	1 A	2 A	M_1	3 A	4 A	M_2	$D_{M_1-M_2}$	5 A	6 A	M_3	$D_{M_1-M_3}$
0-3 hrs.	*	*	-	*	*	-	-	*	*	-	-
1 day	0.45	0.44	0.44	0.44	0.42	0.43	0.01	0.33	0.41	0.37	0.07
3 days	0.70	0.74	0.72	0.70	0.77	0.73	0.01	0.80	0.80	0.80	0.08
9	0.86	0.83	0.84	0.85	0.80	0.82	0.02	0.82	0.87	0.84	0
35	0.90	0.85	0.87	0.86	0.85	0.85	0.02	0.85	0.85	0.85	0.02

- = no determination made.
* = less than 0.01 per cent lactic acid.
A = per cent lactic acid.
M_1 = average acidity values of flasks 1, 2.

M_2 = average acidity values of flasks 3, 4.

M_3 = average acidity values of flasks 5, 6.

$D_{M_1-M_2}$ = difference in acidity between M_1 and M_2 .

$D_{M_1-M_3}$ = difference in acidity between M_1 and M_3.

Flasks 1, 2 = control, nothing added.
Flasks 3, 4 = 0.5% sucrose, added.
Flasks 5, 6 = 1.0% sucrose, added.

TABLE 7 - Continued

Time After Brining	No. of Flask															
	7 A	8 A	M_4	$D_{M_1-M_4}$	9 A	10 A	M_5	$D_{M_1-M_5}$	11 A	12 A	M_6	$D_{M_1-M_6}$	13 A	14 A	M_7	$D_{M_1-M_7}$
0-3 hrs.	*	*	-	-	*	*	-	-	*	*	-	-	*	*	-	-
1 day	0.36	0.42	0.39	0.05	0.38	0.39	0.38	0.06	0.54	0.60	0.51	0.13	0.61	0.63	0.62	0.18
3 days	0.80	0.75	0.77	0.05	0.72	0.71	0.71	0.01	1.01	1.02	1.01	0.29	1.02	1.02	1.02	0.50
9	0.83	0.87	0.85	0.01	0.89	0.82	0.85	0.02	1.31	1.30	1.30	0.46	1.31	1.30	1.30	0.46
35	0.89	0.89	0.89	0.02	0.91	0.87	0.89	0.02	1.32	1.40	1.36	0.49	1.33	1.41	1.37	0.50

M_4 = average acidity values of flasks 7, 8.

M_5 = average acidity values of flasks 9, 10.

M_6 = average acidity values of flasks 11, 12.

M_7 = average acidity values of flasks 13, 14.

$D_{M_1-M_4}$ = difference in acidity between M_1 and M_4.

$D_{M_1-M_5}$ = difference in acidity between M_1 and M_5.

$D_{M_1-M_6}$ = difference in acidity between M_1 and M_7.

Flasks 7, 8 = 0.0025% cystine, 0.0025% tryptophane, added.
Flasks 9, 10 = 0.0025% cystine, 0.0025% tryptophane, 1.0% sucrose, added.
Flasks 11, 12 = 0.821% corn steep liquor, added.
Flasks 13, 14 = 0.821% corn steep liquor, 1.0% sucrose, added.

TABLE 8

PER CENT REDUCING SUGARS[1] IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Fourth Flask Experiment

Time	Per Cent Invert Sugar													
	No. of Flask													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 hrs.	1.304	1.264	1.894	1.959	2.230	2.230	1.294	1.294	2.500	2.368	1.324	1.344	2.275	2.230
35 days	0.368	0.395	0.901	0.988	1.348	1.369	0.350	0.332	1.405	1.354	*	*	0.925	0.874
Reducing sugars utilized	0.936	0.869	0.993	0.971	0.882	0.861	0.944	0.962	1.095	1.014	1.279	1.279	1.350	1.356

[1]Reducing sugars expressed as grams invert sugar per 100 ml of brine.

* = less than 0.045 grams invert sugar per 100 ml of brine.

TABLE 9

PER CENT REDUCING SUGARS[1] IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	Per Cent Invert Sugar											
	No. of Barrel											
	1	2	3	4	5	6	7	8	9	10	11	12
0-3 hrs.	*	1.254	*	1.991	*	0.673	*	*	2.089	*	*	0.687
2 days	0.331	1.269	0.395	1.730	0.436	0.820	0.318	0.318	1.862	0.450	0.423	0.782
4	0.612	1.558	0.782	1.964	0.715	-	-	-	-	-	-	-
6	1.008	1.697	1.013	1.964	0.882	1.234	0.963	0.927	2.000	0.906	1.037	1.132
8	0.821	1.329	1.637	1.810	2.144	1.359	0.621	0.714	1.835	0.327	0.668	0.732
9	0.777	1.003	1.029	1.529	2.024	0.791	0.593	0.782	1.316	*	0.165	0.418
11	0.725	0.615	0.512	0.968	0.646	0.418	0.502	0.427	0.712	*	*	0.268
13	0.336	0.386	0.469	0.624	-	0.422	0.361	0.295	0.515	*	*	-
15	0.091	-	-	-	0.407	0.317	0.055	0.109	-	*	*	0.305
25	*	0.151	0.114	0.241	0.250	*	*	*	0.136	*	*	*
33	*	0.165	0.138	0.173	0.290	*	*	*	0.147	*	*	*
41	*	0.056	0.062	0.080	0.069	*	*	*	0.122	*	*	*

[1]Reducing sugars expressed as grams invert sugar per 100 ml of brine.

- = no determination made.
* = less than 0.045 grams invert sugar per 100 ml of brine.

Effect of sugar on total and acid-forming bacteria counts. In addition to the study of the effect of sugar on brine acidity, observations were also made on the total and acid-forming bacteria counts (Tables 10 and 13). To determine if there is a difference in bacteria counts as a result of addition of sugar, it was necessary at first to study the differences that normally occur between duplicate lots. Thus in 1953, it was possible to compare barrels 1, 3, 5, 8 and 11, only during the first week of fermentation since these barrels did not receive any additions during this period. Maximum differences in the acid-producing bacteria counts, expressed as log. of count, are recorded in Table 12. The greatest difference observed was 1.2. In 1954, comparison in the same manner between individual barrels within one treatment indicates that the greatest variation, i.e., D_1 or D_2, was 1.1 (Table 13). Since variations between individual barrels might be greater if comparison was made between similar treatments performed in different seasons, a study of the differences occurring between the 1953 and the 1954 fermentations was carried out. Results in Table 14 indicate that the largest difference in the log. of acid-forming bacteria counts was 1.4. Thus, it was safe to assume that if a specific treatment resulted in a difference of 1.5 (32 times greater) or more over the control lot, the difference is significant.

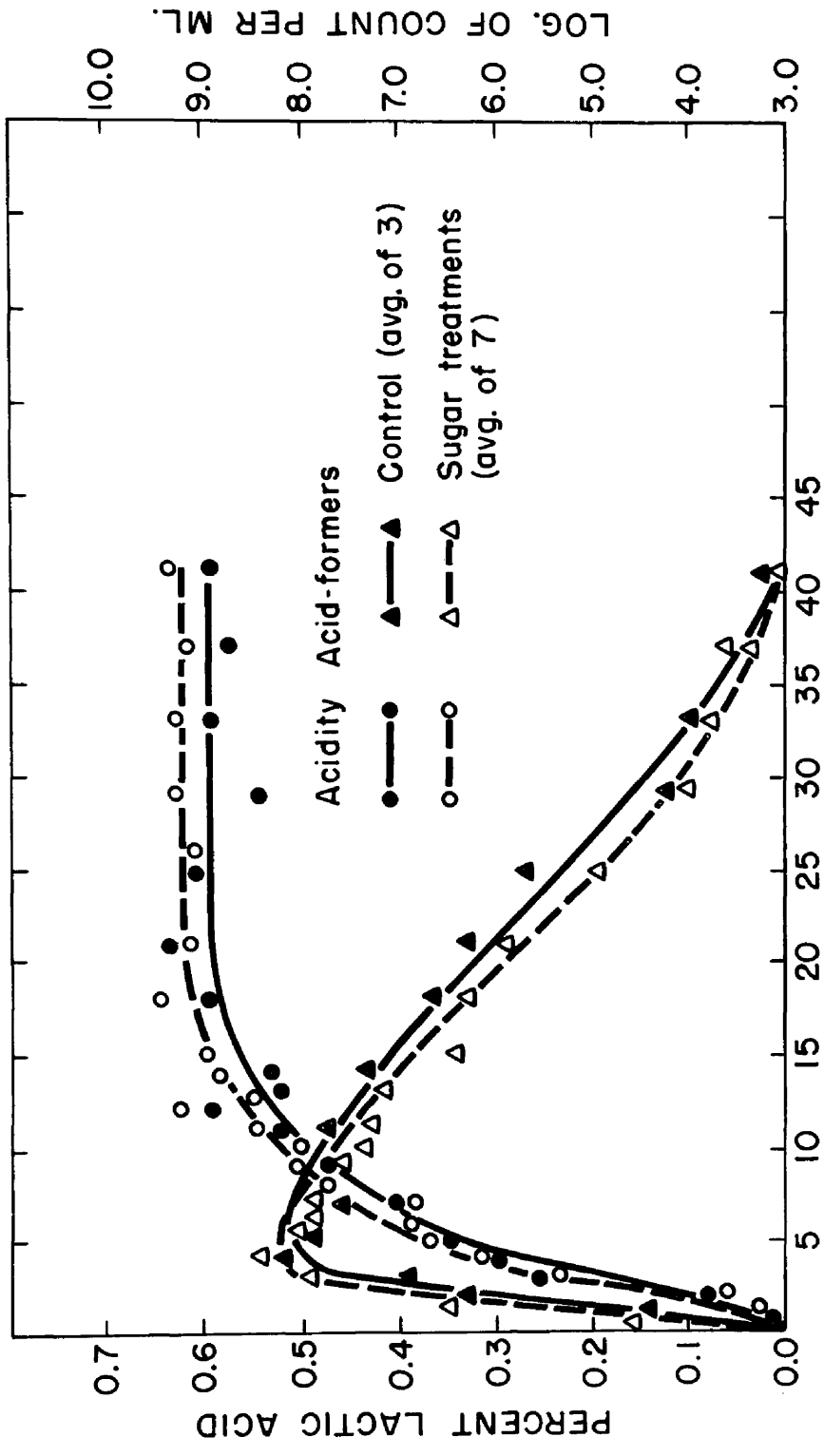

Figure 1. Influence of addition of sugar to brines of semi-commercial cucumber fermentations on lactic acid and acid-forming bacteria.

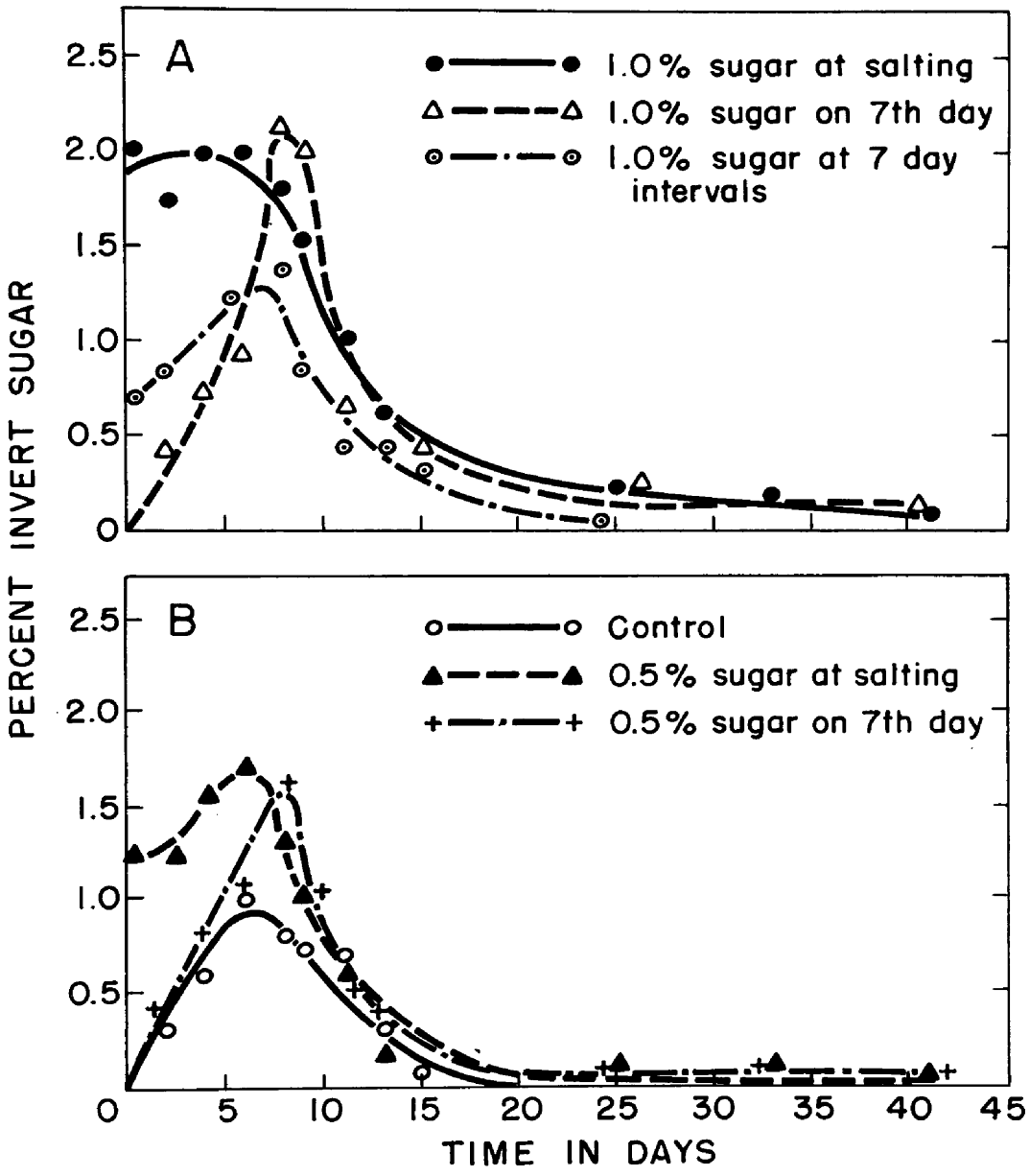

Figure 2. Influence of addition of sugar to brines of semicommercial cucumber fermentations on per cent invert sugar.

TABLE 10

EFFECT OF THE SUGAR TREATMENTS ON TOTAL AND ACID-FORMING BACTERIA COUNTS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel											
	1		2		3		4		5		6	
	T	A	T	A	T	A	T	A	T	A	T	A
0-3 hrs.	0.50	(0.001)	0.72	0.001	0.45	(0.001)	0.30	(0.001)	0.40	(0.001)	0.50	(0.001)
1 day	0.45	0.030	0.45	0.021	0.20	0.011	0.61	0.031	0.70	0.030	0.56	0.04
2 days	2.87	2.70	2.50	2.50	0.74	0.51	2.50	0.55	1.50	1.90	2.30	2.50
3	9.50	7.00	136.0	152.0	8.50	8.00	123.0	127.0	3.80	6.50	145.0	150.00
4	70.00	76.00	125.0	174.0	70.0	85.0	88.5	86.0	33.7	19.00	120.0	143.00
5	46.20	16.20	112.0	95.0	97.0	50.0	67.0	50.0	36.0	16.30	175.0	168.00
6	29.50	24.00	86.0	109.0	95.0	95.0	-	40.0	15.0	10.30	95.0	113.00
7	13.20	10.00	69.0	58.0	40.0	24.0	16.1	14.5	1.90	8.10	47.0	45.00
8	-	-	-	-	25.0	25.0	-	-	1.40	2.30	8.1	18.00
9	4.20	4.00	39.0	56.0	50.0	41.0	13.0	13.0	1.00	5.00	17.60	27.00
10	-	-	-	-	46.5	40.0	-	-	3.20	5.90	10.20	18.00
11	2.20	2.30	26.0	24.0	25.0	38.0	9.5	17.0	1.70	1.50	13.10	13.30
13	1.10	1.00	22.6	20.0	15.5	15.5	30.3	25.0	1.30	1.50	1.80	5.70
15	0.90	0.75	4.5	2.2	0.90	0.70	3.6	4.2	2.30	2.30	1.40	2.20
16	-	-	-	-	-	-	-	-	-	-	1.00	1.60
17	-	-	-	-	-	-	-	-	-	-	0.37	0.30
18	0.35	0.32	0.20	0.15	0.53	0.51	0.23	0.25	0.21	0.25	0.13	0.20
21	0.30	0.35	0.07	0.07	0.20	0.15	0.04	0.05	0.12	0.02	0.06	0.10
25	0.04	0.04	0.01	0.01	0.014	0.016	0.01	0.02	0.10	0.021	0.030	0.05
29	(0.001)	0.001	0.001	0.001	0.004	0.003	0.01	0.01	(0.001)	(0.019) 0.09	0.005	0.006
33	(0.001)	0.002	0.002	0.001	0.005	0.003	0.002	0.002	(0.001)	(0.001)	0.001	0.005
37	(0.001)	(0.001)	0.003	0.001	0.002	(0.001)	0.003	0.002	(0.001)	(0.001)	0.003	0.001
41	(0.001)	0.0016	(0.001)	0.001	0.001	0.001	0.003	0.002	(0.001)	(0.001)	0.004	0.002

- = no determination made.
T = total bacteria count x 10^6/ml
A = acid-forming bacteria count x 10^6/ml
(0.001) = less than 1000 organisms/ml.

TABLE 11

DIFFERENCE IN THE LOG. OF ACID-FORMING BACTERIA COUNTS WHEN DIFFERENT SUGAR ADDITIONS WERE APPLIED TO SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel										
	1 log.	2 log.	D_{1-2}	3 log.	D_{1-3}	4 log.	D_{1-4}	5 log.	D_{1-5}	6 log.	D_{1-6}
0-3 hrs.	(3.0)	3.0	0	(3.0)	0	(3.0)	0	(3.0)	0	(3.0)	0
1 day	4.4	4.3	0.1	4.0	0.4	4.4	0	4.4	0	4.6	0.2
2 days	6.4	6.3	0.1	5.7	0.7	5.7	0.7	6.2	0.2	6.3	0.1
3	6.8	8.1	1.3	6.9	0.1	8.1	1.3	6.8	0	8.1	1.3
4	7.8	8.2	0.4	7.9	0.1	7.9	0.1	7.2	0.6	8.1	1.3
5	7.2	7.9	0.7	7.6	0.4	7.6	0.4	7.2	0	8.2	1.0
6	7.3	8.0	0.7	7.9	0.6	7.6	0.3	7.0	0.3	8.0	1.7
7	7.0	7.7	0.7	7.3	0.3	7.1	0.1	6.9	0.1	7.6	0.6
8	-	-	-	7.3	-	-	-	6.3	-	7.2	-
9	6.6	7.7	1.1	7.6	1.0	7.1	0.5	6.6	0	7.4	0.8
10	-	-	-	7.6	-	-	-	6.7	-	7.2	-
11	6.3	7.3	1.0	7.5	1.2	7.2	0.9	6.1	0.2	7.1	0.8
13	6.0	7.3	1.3	7.1	1.1	7.3	1.3	6.1	0.1	6.7	0.7
15	5.8	6.3	0.5	5.8	0	5.6	0.2	6.3	0.5	6.3	0.5
16	-	-	-	-	-	-	-	-	-	6.2	-
17	-	-	-	-	-	-	-	-	-	5.4	-
18	5.5	5.1	0.4	5.7	0.2	5.3	0.2	5.3	0.2	5.3	0.2
21	5.5	4.8	1.3	5.1	0.4	4.6	0.9	4.3	1.2	5.0	0.5
25	4.6	4.0	0.6	4.2	0.4	4.3	0.3	4.3	0.3	4.6	0
29	3.0	3.0	0	3.5	0.5	4.0	1.0	4.2	1.2	3.7	0.7
33	3.3	3.0	0.3	3.4	0.1	3.3	0	(3.0)	0.3	3.6	0.3
37	(3.0)	3.0	0	(3.0)	0	3.3	0.3	(3.0)	0	3.0	0
41	3.2	3.0	0.2	3.0	0.2	3.3	0.1	(3.0)	0.2	3.3	0.1

- = no determination made.
D = difference in the log. of acid-forming bacteria count between the specific treatment and the control.
log. = logarithm of acid-forming bacteria count.

TABLE 12

DIFFERENCE IN THE LOG. OF ACID-FORMING BACTERIA COUNTS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS DURING THE FIRST WEEK

1953 Fermentations

Time After Brining	No. of Barrel					
	1 log.	3 log.	5 log.	8 log.	11 log.	D
0-3 hrs.	(3.0)	(3.0)	(3.0)	(3.0)	(3.0)	0
1 day	4.4	4.0	4.4	4.3	5.0	1.0
2 days	6.4	5.7	6.2	6.4	6.9	1.2
3	6.8	6.9	6.8	7.9	7.9	1.1
4	7.8	7.9	7.2	8.0	7.8	0.8
5	7.2	7.6	7.2	8.3	7.8	1.1
6	7.3	7.9	7.0	7.9	7.7	0.9
7*	7.0	7.1	6.9	7.8	7.3	0.9

* = counts were made before applying the specific treatment.
D = maximum difference between barrels 1, 3, 5, 8 and 11.
(3.0)= less than 1000 organisms per ml of brine. The log. of count was considered equal to 3.0 when comparative differences were studied.
log.= logarithm of acid-forming bacteria count.

TABLE 13

EFFECT OF THE ADDITION OF SUGAR ON TOTAL AND ACID-FORMING BACTERIA COUNTS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1954 Fermentations

Time After Brining	No. of Barrel														
	1			2				3			4				
	T	A	$\log_1$	T	A	$\log_2$	D_1	T	A	$\log_3$	T	A	$\log_4$	D_2	D_3
0-3 hrs.	(0.001)	(0.001)	(3)	(0.001)	(0.001)	(3)	0	(0.001)	(0.001)	(3)	(0.001)	(0.001)	(3)	0	0
2 days	4.0	2.5	6.3	1.00	0.87	5.9	0.4	7.4	7.6	6.8	0.60	0.80	5.9	0.9	0.9
3	11.0	8.0	6.9	16.0	8.4	6.9	0	61.0	47.0	7.6	135.0	126.0	8.0	0.4	1.1
4	228.0	245.0	8.3	185.0	189.0	8.2	0.1	460.0	600.0	8.7	400.0	630.0	8.7	0	0.5
5	135.0	133.0	8.1	87.0	111.0	8.0	0.1	341.0	330.0	8.5	132.0	160.0	8.2	0.3	0.5
7	62.0	62.6	7.7	75.0	79.0	7.8	0.1	260.0	247.0	8.3	44.0	74.0	7.8	0.5	0.6
8	-	-	-	-	-	-	-	153.0	187.0	8.2	81.0	100.0	8.0	0.2	-
9	46.0	34.0	7.5	125.0	118.0	8.0	0.5	124.0	131.0	8.1	35.0	50.0	7.6	0.5	0.6
12	16.0	10.0	7.0	108.0	100.0	8.0	1.0	137.0	138.0	8.1	29.0	16.0	7.2	0.9	1.1
14	7.0	8.0	7.9	40.0	35.2	7.5	0.6	64.0	46.5	7.6	10.5	7.9	6.8	0.8	0.8
18	6.0	5.1	6.7	10.5	9.1	6.9	0.2	17.0	9.0	6.9	1.10	1.30	6.1	0.8	0.8
21	2.0	2.5	6.3	2.5	2.9	6.4	0.1	3.1	3.6	6.5	0.60	0.60	5.7	0.8	0.8
25	0.60	0.42	5.6	0.50	1.1	6.0	0.4	0.40	0.35	5.5	0.10	0.10	5.0	0.5	1.0
29	0.10	0.024	4.3	0.20	0.028	4.4	0.1	0.23	0.017	4.2	0.02	0.02	4.3	0.1	0.2
33	0.04	0.020	4.3	0.02	0.010	4.0	0.3	0.034	0.028	4.4	0.03	0.009	3.9	0.5	0.5
37	0.01	0.005	3.6	0.003	0.004	3.6	0	0.027	0.030	4.4	0.01	0.002	3.3	1.1	1.1
41	0.01	0.003	3.4	0.002	(0.001)	(3)	0.4	0.005	0.004	3.6	0.003	(0.001)	3.0	0.6	0.6

- = no determination made.

(0.001) = less than 1000 organisms/ml of brine, The log. of count was considered equal to (3) when comparative differences were studied.

T = total bacteria count x 10^6/ml.

A = total acid-forming bacteria count x 10^6/ml.

$\log_1$, $\log_2$, $\log_3$, $\log_4$ = logarithm of acid-forming bacteria count in barrels 1, 2, 3 and 4.

D_1 = difference between $\log_1$, $\log_2$.

D_2 = difference between $\log_3$, $\log_4$.

D_3 = maximum difference between $\log_1$, $\log_2$, $\log_3$, $\log_4$.

TABLE 14

DIFFERENCE IN THE LOG. OF ACID-FORMING BACTERIA COUNTS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS PREPARED IN TWO DIFFERENT SEASONS

1953 and 1954 Fermentations

Time After Brining	No. of Barrel 1(53) log.	1(54) log.	2(54) log.	D_1	3(53) log.	3(54) log.	4(54) log.	D_2
0-3 hrs.	(3.0)	(3.0)	(3.0)	0	(3.0)	(3.0)	(3.0)	0
2 days	6.4	6.3	5.9	0.5	5.7	6.8	5.9	1.1
3	6.8	6.9	6.9	0.1	6.9	7.6	8.0	1.1
4	7.8	8.3	8.2	0.5	7.9	8.7	8.7	0.8
5	7.2	8.1	8.0	0.9	7.6	8.5	8.2	0.9
7	7.0	7.7	7.8	0.8	7.3	8.3	7.8	1.0
8	-	-	-	-	7.3	8.2	8.0	0.9
9	6.6	7.5	8.0	1.4	7.6	8.1	7.6	0.5
18	5.5	6.7	6.9	1.4	5.7	6.9	6.1	1.2
21	5.5	6.3	6.4	0.9	5.1	6.5	5.7	1.4
25	4.6	5.6	6.0	1.4	4.2	5.5	5.0	1.3
29	3.0	4.3	4.4	1.4	3.5	4.2	4.3	0.8
33	3.3	4.3	4.0	1.0	3.4	4.4	3.9	1.0
37	(3.0)	3.6	3.6	0.6	(3.0)	4.4	3.3	1.4
41	3.2	3.4	(3.0)	0.4	3.0	3.6	(3.0)	0.6

- = no determination made.
log.= logarithm of acid-forming bacteria counts.
D_1= maximum difference in log. of acid-forming bacteria count between barrels 1(53), 1(54), 2(54).
D_2= maximum difference in log. of acid-forming bacteria count between barrels 3(53), 3(54), 4(54).
(3.0)= less than 1000 organisms per ml of brine. The log. of count was considered equal to 3.0 when comparative differences were studied.

TABLE 15

EFFECT OF THE SUGAR TREATMENTS ON L. PLANTARUM[1] COUNTS IN BRINES OF LABORATORY CUCUMBER FERMENTATION:

Third Flask Experiment

Time After Brining	No. of Flask 1	log.	2	log.	3	log.	4	log.	5	log.	6	log.
0-3 hrs.	120.0	8.0	122.0	8.0	125.0	8.0	115.0	8.0	117.0	8.0	120.0	8.0
2 days	3380.0	9.5	2150.0	9.3	1860.0	9.2	2650.0	9.4	3190.0	9.5	2750.0	9.4
6	1770.0	9.2	2810.0	9.4	1240.0	9.0	2030.0	9.3	1250.0	9.0	1140.0	9.0
11	87.0	7.9	102.0	8.0	64.0	7.8	58.0	7.7	151.0	8.1	41.0	7.6
17	0.6	5.7	0.4	5.6	0.8	5.9	1.1	6.0	2.5	6.3	0.7	5.8
25	0.01	4.0	0.01	4.0	-	-	0.01	4.0	0.01	4.8	0.02	4.3

Time After Brining	No. of Flask 7	log.	8	log.	9	log.	10	log.
0-3 hrs.	122.0	8.0	112.0	8.0	120.0	8.0	125.0	8.0
2 days	2130.0	9.3	2880.0	9.4	2700.0	9.4	2510.0	9.3
6	1220.0	9.0	1040.0	9.0	1190.0	9.0	850.0	8.9
11	63.0	7.7	124.0	8.0	54.0	7.7	90.0	7.9
17	3.1	6.4	2.3	6.3	1.9	6.2	2.4	6.3
25	0.1	5.0	0.03	4.5	0.1	5.0	0.04	4.6

- = no determination made.

log. = logarithm of counts

[1]Counts x 10^6/ml.

TABLE 16

EFFECT OF THE SUGAR TREATMENTS ON L. PLANTARUM[1] COUNTS IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Fourth Flask Experiment

Time After Brining	No. of Flask											
	1	log.	2	log.	3	log	4	log.	5	log.	6	log.
0-3 hrs.	90.0	7.9	92.0	7.9	100.0	8.0	88.0	7.9	100.0	8.0	90.0	7.9
1 day	1760.0	9.2	1940.0	9.2	2040.0	9.3	2220.0	9.3	1300.0	9.1	1540.0	9.1
3 days	1540.0	9.1	2060.0	9.3	1780.0	9.2	2180.0	9.3	1360.0	9.1	2080.0	9.3
9	1.1	6.0	2.1	6.3	1.2	6.0	1.9	6.2	1.8	6.2	1.4	6.1
35	0.003	3.5	0.002	3.3	0.005	3.7	0.003	3.5	0.005	3.7	0.005	3.7

log. = logarithm of counts

[1]Counts x 10^6/ml.

In the 1953 fermentations, when 0.5 or 1.0 per cent sugar was added at the time of salting, or on the 7th day, or in small portions at 7-day intervals throughout the fermentation, there was no indication of an appreciable increase in the acid-forming bacterial population (Table 11). Slight variations in counts, which did not exceed the range of differences that might occur between individual barrels, were observed. Thus, in barrel 5 the acid-producing bacteria counts were 8.1 x 10^6/ml on the 7th day, whereas the control barrel showed a population of 10 x 10^6/ml. After adding 1.0 per cent sugar, the counts on the 9th day were 5 x 10^6/ml and 4 x 10^6/ml for barrels 5 and 1 respectively. No significant difference as a result of the treatment is observed. Also, in barrel 6, addition of the second portion of sugar on the 7th day of fermentation caused no significant increase in counts over that of the control, i.e., 27 x 10^6/ml as compared to 4 x 10^6/ml on the 9th day. When the third portion of sugar was added, no increase in population occurred. On the contrary, the counts continued to decrease gradually for the rest of the examination period. Also in 1954, the maximum difference in the log. of acid-forming bacteria counts between the sugar and no-sugar treatments, i.e., D_3, did not exceed 1.1, which is not a significant difference (Table 13).

Since no difference in the acid-forming bacteria counts was observed between the different concentrations of sugar,

the average counts for all sugar treatments under semicommercial conditions, are presented in Fig. 1. It is evident from this graph that the population of these organisms in general, increased rapidly after brining and reached a peak within 5 to 6 days. During the second week of fermentation the numbers remained somewhat high, but declined during the third week with very low population observed after 41 days.

The third and fourth laboratory experiments carried out under the best of conditions indicate no significant effect on the numbers of L. plantarum as a result of adding different amounts of sugar (Tables 15 and 16). In one instance, there was a significant increase in the population of this organism over the control, e.g., flask 9 showed a population of 0.10×10^6/ml on the 25th day as compared to 0.01×10^6/ml in the control (Table 15). However, this was not true for flasks 8 and 10 which received the same treatment as flask 9.

The average counts of L. plantarum for all the sugar treatments as well as the control fermentations, carried out in the third laboratory experiment, is shown in Fig. 4, part A.

There was no need for determining the total bacterial count in the laboratory experiments since the flasks were inoculated with L. plantarum only.

Effect of sugar on yeast populations. A study of the differences in yeast populations between similar barrels

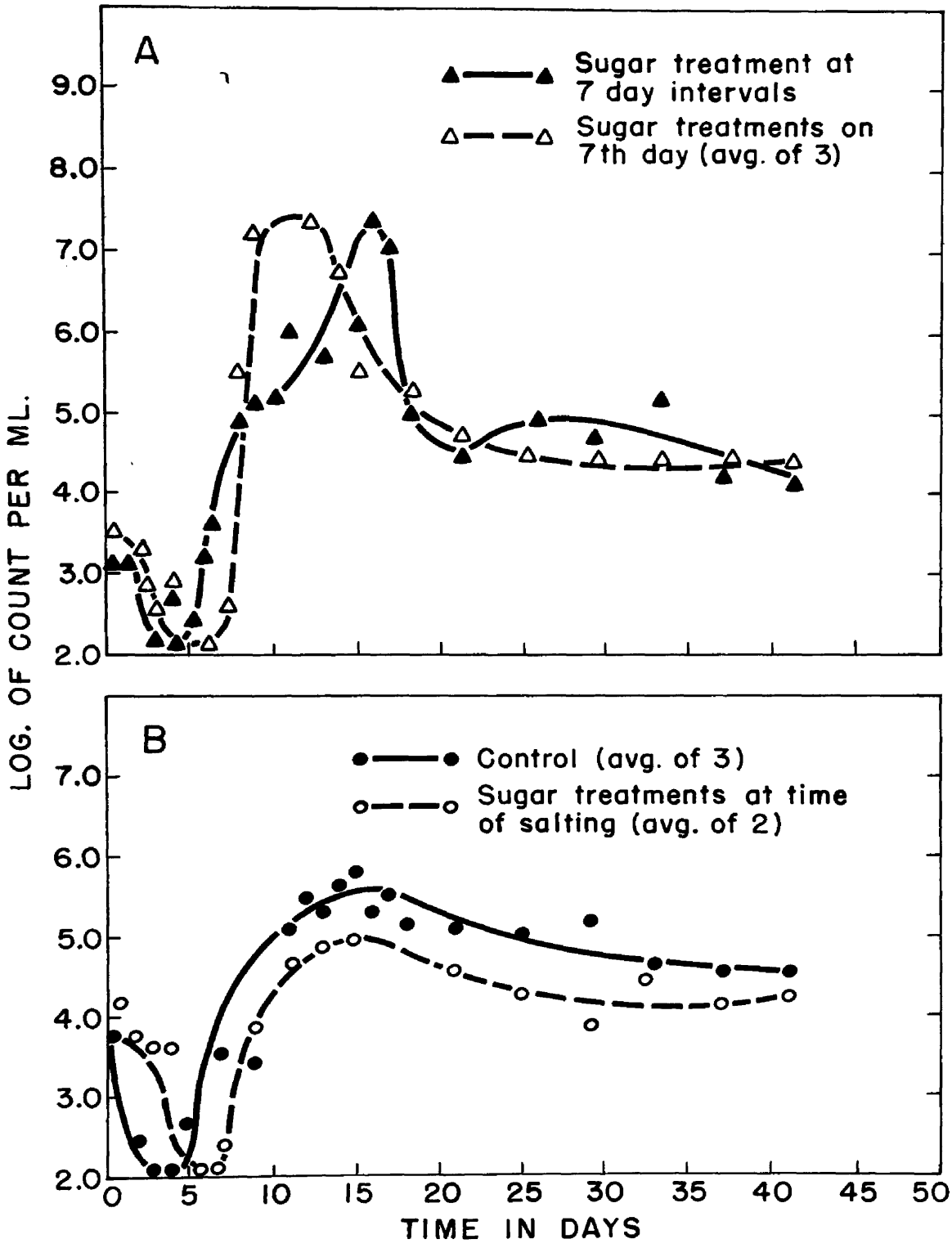

Figure 3. Influence of addition of sugar to brines of semicommercial cucumber fermentations on yeasts.

TABLE 17

EFFECT OF DIFFERENT SUGAR TREATMENTS ON YEAST POPULATIONS[1] IN BRINES OF SEMICOMMERCIAL FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel							
	1	$\log_1$	2	$\log_2$	d_{1-2}	3	$\log_3$	d_{1-3}
0-3 hrs.	3.0	3.4	0.80	2.9	0.5	1.0	3.0	0.4
1 day	1.5	3.1	0.40	2.6	0.5	1.0	3.0	0.1
2 days	0.7	2.8	(0.10)	(2.0)	0.8	0.3	2.4	0.4
3	0.2	2.3	(0.10)	(2.0)	0.3	(0.1)	(2.0)	0.3
4	(0.1)	(2.0)	0.60	2.7	0.7	0.4	2.6	0.6
5	0.1	2.0	0.10	2.0	0	0.2	2.3	0.3
6	0.1	2.0	0.10	2.0	0	(0.1)	(2.0)	0
7	1.0	3.0	0.40	2.6	0.4	(0.1)	(2.0)	1.0
8	0.1	2.0	-	-	-	10.0	4.0	2.0
9	0.4	2.6	12.50	4.0	1.4	6900.0	6.8	4.2
10	1.0	3.0	-	-	-	1800.0	6.2	3.2
11	150.0	5.1	10.10	4.0	1.1	2200.0	6.3	1.2
13	250.0	5.3	88.0	4.9	0.4	46.0	4.6	0.7
15	750.0	5.8	51.0	4.7	1.1	28.0	4.4	1.4
16	221.0	5.3	-	-	-	-	-	-
17	380.0	5.5	-	-	-	-	-	-
18	250.0	5.3	12.8	4.1	1.2	17.5	4.2	1.1
21	120.0	5.0	7.0	3.8	1.2	20.0	4.3	0.7
25	120.0	5.0	5.4	3.7	1.3	3.1	3.4	1.6
29	7.0	3.8	4.5	3.6	0.2	19.8	4.2	0.4
33	17.5	4.2	76.0	4.2	0	40.0	4.6	0.4
37	22.6	4.3	6.7	3.8	0.5	11.0	4.0	0.3
41	23.2	4.3	10.6	4.0	0.3	6.5	3.8	0.5

[1] counts $x10^3$/ml

- = no determination made

log = logarithm of yeast count per ml of brine

d_{1-2} = difference between $\log_1$, $\log_2$.

d_{1-3} = difference between $\log_1$, $\log_3$.

(2.0) = less than 100 organisms per ml of brine. The log. of count was considered equal to 2.0 when comparative differences were studied.

TABLE 17 - Continued

Time After Brining	No. of Barrel								
	4	$\log_4$	d_{1-4}	5	$\log_5$	d_{1-5}	6	$\log_6$	d_{1-6}
0-3 hrs.	3.0	3.4	0	3.9	3.5	0.1	1.5	3.1	0.3
1 day	28.0	4.4	1.3	1.3	3.1	0	1.5	3.1	0
2 days	11.0	4.0	1.2	1.9	3.2	0.4	9.0	3.9	1.1
3	10.0	4.0	1.7	(0.10)	(2.0)	0.3	0.1	2.0	0.3
4	7.4	3.8	1.8	(0.10)	(2.0)	0	0.4	2.6	0.6
5	0.1	2.0	0	(0.10)	(2.0)	0	0.2	2.3	0.3
6	0.1	2.0	0	(0.10)	(2.0)	0	1.5	3.1	1.1
7	0.1	2.0	1.0	(0.10)	(2.0)	1.0	3.5	3.5	0.5
8	-	-	-	7.7	3.8	1.8	65.0	4.8	2.8
9	0.5	2.6	0	38000.0	7.5	4.9	100.0	5.0	2.4
10	-	-	-	3500.0	6.5	3.5	125.0	5.0	2.0
11	79.0	4.8	0.3	100.0	5.0	0.1	800.0	5.9	0.8
13	85.0	4.9	0.4	140.0	5.1	0.2	500.0	5.6	0.3
15	132.0	5.1	0.7	880.0	5.9	0.1	1000.0	6.0	0.2
16	-	-	-	-	-	-	21000.0	7.3	2.0
17	-	-	-	-	-	-	11000.0	7.0	1.5
18	13.4	4.1	1.2	130.0	5.1	0.2	88.0	4.9	0.4
21	70.0	4.8	0.2	61.0	4.7	0.3	30.0	4.4	0.6
25	27.3	4.4	0.6	90.0	4.9	0.1	90.0	4.9	0.1
29	10.8	4.0	0.2	123.0	5.0	1.2	45.0	4.6	0.8
33	52.0	4.7	0.5	57.0	4.7	0.5	160.0	5.2	1.0
37	20.6	4.3	0	23.0	4.3	0	19.0	4.2	0.1
41	41.0	4.6	0.3	25.0	4.3	0	11.0	4.0	0.3

d_{1-4} = difference between $\log_1$, $\log_4$.

d_{1-5} = difference between $\log_1$, $\log_5$.

d_{1-6} = difference between $\log_1$, $\log_6$.

TABLE 18

DIFFERENCE IN THE LOG. OF YEAST COUNTS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS DURING THE FIRST WEEK

1953 Fermentations

Time After Brining	No. of Barrel					
	1 log.	3 log.	5 log.	8 log.	11 log.	D
0-3 hrs.	3.4	3.0	3.5	3.9	3.3	0.9
1 day	3.1	3.0	3.1	3.4	3.1	0.4
2 days	2.8	2.4	3.2	3.6	2.3	1.4
3	2.3	(2.0)	(2.0)	(2.0)	2.0	0.3
4	(2.0)	2.6	(2.0)	(2.0)	2.0	0.6
5	2.0	2.3	(2.0)	(2.0)	2.0	0.3
6	2.0	(2.0)	(2.0)	(2.0)	3.2	1.2
7*	3.0	(2.0)	(2.0)	(2.0)	3.2	1.2

*Counts were made before applying the specific treatment.

log. = logarithm of yeast count.

D = maximum differences in the log. of yeast count between barrels 1, 3, 5, 8, and 11.

(2.0) = counts less than 100 organisms per ml of brine. The log. of count was considered equal to 2.0 when comparative differences were studied.

TABLE 19

EFFECT OF SUGAR ON YEAST POPULATIONS[1] IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1954 Fermentations

Time After Brining	No. of Barrel										
	1	$\log_1$	2	$\log_2$	d_{1-2}	3	$\log_3$	4	$\log_4$	d_{3-4}	D
0-3 hrs.	5.60	3.7	13.7	4.1	0.4	3.50	3.5	4.20	3.6	0.1	0.6
2 days	(0.10)	(2.0)	(0.10)	(2.0)	0	0.50	2.6	(0.10)	(2.0)	0.6	0.6
3	(0.10)	(2.0)	(0.10)	(2.0)	0	0.70	2.8	0.50	2.6	0.2	0.8
4	(0.10)	(2.0)	(0.10)	(2.0)	0	1.00	3.0	1.40	3.1	0.1	1.1
5	0.40	2.6	0.70	2.8	0.2	1.50	3.1	6.90	3.8	0.7	1.2
7	4.20	3.6	4.60	3.6	0	4.60	3.6	10.00	4.0	0.4	0.4
8	-	-	-	-	-	112.0	5.1	1060.0	6.0	0.9	-
9	34.0	4.5	56.0	4.7	0.2	6000.0	6.7	7000.0	6.8	0.1	2.3
12	172.0	5.2	410.0	5.6	0.4	10000.0	7.0	33000.0	7.5	0.5	2.3
14	490.0	5.6	380.0	5.5	0.1	8300.0	6.9	3900.0	6.5	0.4	1.4
18	110.0	5.0	41.0	4.6	0.4	320.0	5.5	240.0	5.3	0.2	0.9
21	227.0	5.3	41.0	4.6	0.7	14.0	4.1	78.0	4.8	0.7	1.2
25	-	-	-	-	-	20.0	4.3	6.0	3.7	0.6	-
29	420.0	5.6	157.0	5.1	0.5	7.0	3.8	4.0	3.6	0.2	1.8
33	59.0	4.7	52.0	4.7	0	7.1	3.8	40.0	4.6	0.8	0.9
37	54.0	4.7	40.0	4.6	0.1	9.2	3.9	36.0	4.5	0.6	0.8
41	16.7	4.2	55.0	4.7	0.5	8.0	3.9	50.0	4.6	0.7	0.8

[1] counts $\times 10^3$/ml

\- = no determination

(0.10) = less than 100 organisms per ml of brine. The log. of count was considered equal to 2.0 when comparative differences were studied.

log. = logarithm of yeast count.

d_{1-2} = difference between $\log_1$ and $\log_2$.

d_{3-4} = difference between $\log_3$ and $\log_4$.

D = maximum difference between $\log_1$, $\log_2$, $\log_3$ and $\log_4$.

TABLE 20

DIFFERENCE IN THE LOG. OF YEAST COUNTS IN BRINES OF SIMILAR LOTS OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS PREPARED IN TWO DIFFERENT SEASONS

1953 and 1954 Fermentations

Time After Brining	No. of Barrel							
	1(53)	1(54)	2(54)		3(53)	3(54)	4(54)	
	log.	log.	log.	D_1	log.	log.	log.	D_2
0-3 hrs.	3.4	3.7	4.1	0.7	3.0	3.5	3.6	0.6
2 days	2.8	(2.0)	(2.0)	0.8	2.4	2.6	(2.0)	0.6
3	2.3	(2.0)	(2.0)	0.3	(2.0)	2.8	2.6	0.8
4	(2.0)	(2.0)	(2.0)	0	2.6	3.0	3.1	1.5
5	2.0	2.6	2.8	0.8	2.3	3.1	3.8	1.5
7	3.0	3.6	3.6	0.6	(2.0)	3.6	4.0	2.0
8	2.8	-	-	-	4.0	5.1	6.0	2.0
9	2.6	4.5	4.7	2.1	6.8	6.7	6.8	0.1
18	5.3	5.0	4.6	0.7	4.2	5.5	5.3	1.3
21	5.0	5.3	4.6	0.7	4.3	4.1	4.8	0.9
25	5.0	-	-	-	3.4	4.3	3.7	0.9
29	3.8	5.6	5.1	1.8	4.2	3.8	3.6	0.6
33	4.2	4.7	4.7	0.5	4.6	3.8	4.6	0.8
37	4.3	4.7	4.6	0.4	4.0	3.9	4.5	0.6
41	4.3	4.2	4.7	0.5	3.8	3.9	4.6	0.8

- = no determination made

D_1 = maximum difference between barrels 1(53), 1(54) and 2(54)

D_2 = maximum difference between barrels 3(53), 3(54) and 4(54)

(2.0) = less than 100 organisms per ml of brine. The log. of count was considered equal to 2.0 when comparative differences were studied.

was carried out in the same manner as in the case of the acid-forming bacteria counts. Tables 18 and 19 indicate that a variation of 1.4 in the log. of yeast counts might occur between two duplicate barrels prepared in one season. However, when comparison was made between similar treatments performed in 1953 and 1954, the log. of yeast counts varied by 2.1 (Table 20). Accordingly, it was assumed that a difference of 2.0 or more in the log of yeast count, i.e., 100 times greater, is significant only if comparison is made between fermentations prepared in one season.

The populations of yeast in 1953 and 1954 are found in Tables 17 and 19. In 1953, the addition of 0.5 or 1.0 per cent sucrose at the time of salting resulted in no significant difference in yeast population over that of the control (Fig. 3, part B). The difference in the log. of yeast counts, i.e., d_{1-2} and d_{1-4}, never exceeded 2.0 (Table 17). However, when 0.5 per cent sugar was added on the 7th day of fermentation, a rapid increase in yeast population was observed. For example, Table 17 shows that in barrel 3 the yeast count was less than 100 organisms per ml of brine on the 7th day. After addition of sugar, yeast numbers rapidly increased and reached a value of 6900 x 10^3/ml on the 9th day. Consequently, there was a difference of 4.2 (1600 times greater) in the log. of yeast counts over that of the control. When 1.0 per cent sucrose was added on the 7th day of fermentation, similar

results were obtained. Thus in both cases, addition of sugar on the 7th day resulted in a rapid increase in yeast population. This was followed by a rapid decline until the populations attained a level similar to that of the control. In the 1954 experiment, similar results were observed (Table 19). Thus the average yeast counts of all fermentations in 1953 and 1954 which received sugar on the 7th day, are presented in Fig. 3, part A.

When the amount representing 1.0 per cent sugar of the weight of cucumbers and brine was divided and added at salting time and at two 7-day intervals, no significant rise in yeast population over that of the control was observed during the first week of fermentation. Fig. 3, part A, shows however, that the second addition of sugar resulted in a significant rise in yeast population. When the last portion of sugar was added on the 14th day, the increase in yeast numbers was doubtful. The log. of yeast counts increased over that of the control by 2.0 and 1.5 on the 16th and 17th days respectively (Table 17). These differences are not greater than 2.0 and therefore are considered insignificant.

In contrast to the acid-forming bacteria, yeasts in general slightly decreased in numbers during the first few days after salting, but started rapid growth after about 7 days, and reached their maximum population in from 10 to 15 days.

Effect of sugar on coliform organisms. The coliform populations in 1953 and 1954 fermentations are presented in Table 21. Although large variations were noted in the numbers present initially in the different fermentations, no coliforms were found after five days. Also, no effect of addition of sugar was observed. It is believed that the coliforms played no significant part in these fermentations. Apparently, they were introduced into the brine by adhering to the surface of the cucumbers, found conditions unfavorable for growth and consequently disappeared. These results are in close agreement with the findings of Costilow and Fabian (8) under laboratory conditions.

TABLE 21

EFFECT OF DIFFERENT SUGAR TREATMENTS ON COLIFORM[1] POPULATIONS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

Time After Brining	No. of Barrel									
	1953 Fermentations						1954 Fermentations			
	1	2	3	4	5	6	1	2	3	4
0-3 hrs.	4300	4300	740	740	1180	92	430	920	220	430
1 day	9200	9200	430	650	2200	1180	-	-	-	-
2 days	430	92	740	920	430	250	9200	9200	4300	9200
3	15	24	430	**	**	24	2500	240	430	4300
4	**	**	15	**	**	**	24	24	43	**
5	**	**	**	**	**	**	**	**	24	**
6	**	**	**	**	**	**	-	-	-	-
7	**	**	**	**	**	**	**	**	**	**
8	-	-	**	-	**	**	**	**	**	**
9	**	**	**	**	**	**	**	**	**	**
10	**	-	**	-	**	**	-	-	-	-
11	**	**	**	**	**	**	-	-	-	-
12	-	-	-	-	-	-	**	**	**	**
13	**	**	**	**	**	**	-	-	-	-
14	-	-	-	-	-	-	**	**	**	**
15	**	**	**	**	**	**	**	**	**	**
16	**	**	**	**	**	**	-	-	-	-
17	**	**	**	**	**	**	-	-	-	-
18	**	**	**	**	**	**	**	**	**	**
21	**	**	**	**	**	**	**	**	**	**
25	**	**	**	**	**	**	**	**	**	**
29	**	**	**	**	**	**	**	**	**	**
33	**	**	**	**	**	**	**	**	**	**
37	**	**	**	**	**	**	**	**	**	**
41	**	**	**	**	**	**	**	**	**	**

[1] No. per ml of brine.

- = no determination made.

** = less than 10 organisms per ml.

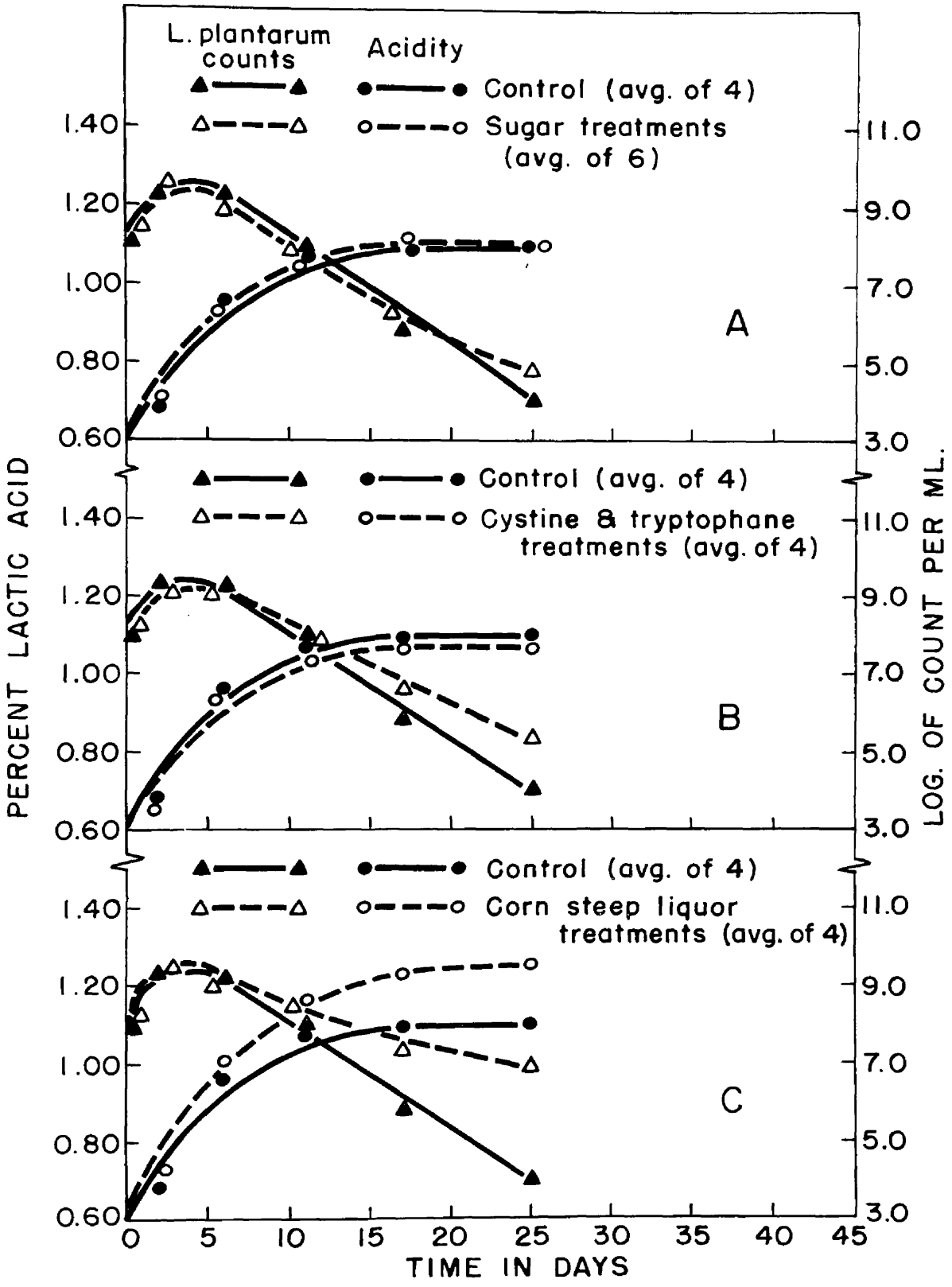

Figure 4. Influence of addition of sugar, addition of cystine and tryptophane, and addition of corn steep liquor to brines of laboratory cucumber fermentations on lactic acid and L. plantarum.

II - Influence of Added Amino Acids on Cucumber Fermentations

Studies by Costilow and Fabian (10) showed that cystine and tryptophane might be reduced during cucumber fermentation to a critical level for the growth of L. plantarum. Therefore, the effect of adding these amino acids to cucumber salt stock was investigated. In 1953, barrel 7 was prepared by adding the amino acids at the time of salting. Barrel 8 was prepared in the same manner except that cystine and tryptophane were added on the 7th day of fermentation. In this way, addition of amino acids at the start and during the active fermentation period was studied. It was then thought that the combined treatment of amino acids and 1.0 per cent sucrose might have an effect on brine acidity as well as on the microbiologic activity. Consequently, barrel 9 was prepared by adding both the amino acids and sugar at the time of salting. Based on the above studies (10), a concentration of 0.0025 per cent of each amino acid was used in this investigation. These concentrations should be more than sufficient to provide the brine microorganisms with plentiful amounts of these amino acids.

Effect of amino acids on lactic acid formation. Results of the effect of the addition of amino acids on lactic acid formation are found in Table 1. It was shown earlier in this study that a variation greater than 0.10 per cent in acid

formation between different treatments should be considered significant. This value was also used in these studies as a basis for comparison between the barrels receiving cystine and tryptophane and the control barrels. In all fermentations examined the amount of acid formed in brines receiving cystine and tryptophane was not appreciably greater than that in the control fermentation. It is true that sometimes an increase of about 0.15 per cent acid over that of the control occurred as in barrel 7 on the 3rd and 18th days. However, this and similar increase in acidity did not last for a considerable period of time, and consequently is of no importance. The flask experiments confirmed these results. It is evident from Tables 6 and 7 that under ideal conditions the difference in brine acidity between all the cystine and tryptophane treatments examined and the control fermentations, were very small and negligible. Therefore, it was not surprising to find that under laboratory conditions, the cystine and tryptophane treatments did not result in more utilization of reducing sugars by L. plantarum (Table 8).

Fig. 4, part B, and Fig. 5 show the average acidity values of all the cystine and tryptophane treatments examined under laboratory and semicommercial conditions respectively.

In 1953, the reducing sugar contents of brines of the cystine and tryptophane fermentations were also studied. Table 9 and Fig. 7, part A show that in both barrels 7 and 8,

the reducing sugar concentrations increased after brining, reached a maximum about the 6th day, thendeclined to a level less than 0.045 per cent invert sugar after 25 days. These results are similar to those obtained in the control fermentation; indicating no faster or greater utilization of reducing sugars as a result of these treatments. When the two amino acids were added in combination with sugar at the time of salting (barrel 9), the concentrations of reducing sugars were considerably higher during the first week. However, they decreased until their level was comparable to that of the control, i.e., 0.515 per cent invert sugar in barrel 9 as compared to 0.336 per cent in the control, on the 13th day.

In general since no greater amounts of acid were formed in all these fermentations, one can only conclude that tryptophane and cystine under the conditions of these experiments, are not limiting factors in acid production.

Effect of cystine and tryptophane on microbial populations. The total bacterial population, the number of acid formers, the number of yeasts and coliforms for each specific treatment are presented in Tables 22 and 23. Fig. 4, part B, and Fig. 5 represent the average acid-forming bacteria counts under laboratory and semicommercial conditions. The average yeast numbers are also presented in Fig 6, part B. It is evident from these studies that no significant rise in the total and acid-forming bacteria populations, as well

TABLE 22

EFFECT OF THE CYSTINE AND TRYPTOPHANE TREATMENTS ON TOTAL AND ACID-FORMING BACTERIA POPULATIONS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel											
	7				8				9			
	T	A	$\log_7$	d_{1-7}	T	A	$\log_8$	d_{1-8}	T	A	$\log_9$	d_{1-9}
0-3 hrs.	0.70	(0.001)	(3.0)	0	0.95	(0.001)	(3.0)	0	0.20	(0.001)	(3.0)	0
1 day	0.65	0.01	4.0	0.4	0.80	0.02	4.3	0.1	0.50	0.04	4.6	0.2
2 days	0.55	0.24	5.3	1.1	2.50	3.00	6.4	0	12.3	15.20	7.1	0.7
3	28.5	25.0	7.3	0.5	170.0	85.0	7.9	1.1	87.0	200.0	8.3	1.5
4	70.0	50.0	7.6	0.2	112.0	115.0	8.0	0.2	74.2	87.0	7.9	0.1
5	65.0	65.0	7.8	0.6	75.0	200.0	8.3	1.1	103.0	100.0	8.0	0.8
6	85.0	22.0	7.3	0	75.0	85.0	7.9	0.6	70.0	70.0	7.8	0.5
7	26.5	24.2	7.3	0.3	70.0	65.0	7.8	0.8	80.0	60.0	7.7	0.7
8	-	-	-	-	20.0	45.0	7.6	-	-	-	-	-
9	17.2	11.5	7.0	0.4	60.0	55.0	7.7	1.1	80.0	85.0	7.9	1.3
10	-	-	-	-	60.0	65.0	7.8	-	-	-	-	-
11	2.2	2.0	6.3	0	70.0	70.0	7.8	1.5	80.0	90.0	7.9	1.4
13	1.7	2.0	6.3	0.3	26.0	30.0	7.4	1.4	30.0	25.0	7.3	1.3
15	0.14	0.27	5.4	0.4	10.5	10.5	7.0	1.2	5.5	8.2	6.9	1.1
18	0.12	0.12	5.0	0.5	0.75	0.95	5.9	0.4	0.10	0.20	5.3	0.2
21	0.07	0.01	4.0	1.5	0.75	0.70	5.8	0.3	0.04	0.08	4.9	0.6
25	0.01	0.002	3.3	1.3	0.23	0.30	5.4	0.8	0.01	0.01	4.0	0.6
29	0.002	0.001	3.0	0	0.04	0.01	4.0	1.0	0.003	0.004	3.6	0.6
33	(0.001)	(0.001)	(3.0)	0.3	0.04	0.01	4.1	0.8	0.005	-	-	-
37	(0.001)	(0.001)	(3.0)	0	0.01	0.006	3.8	0.8	0.005	0.012	4.0	1.0
41	(0.001)	(0.001)	3.7	0.5	0.01	0.005	3.6	0.4	0.002	0.014	4.1	0.9

- = no determination made.
A = acid-forming bacteria count x 10^6/ml.
T = total bacteria x 10^6/ml.

log. = logarithm of acid-forming bacteria count.
d = difference in the log. of acid-forming bacteria count between the control and the specific treatment.

TABLE 23

EFFECT OF THE CYSTINE AND TRYPTOPHANE TREATMENTS ON YEAST AND COLIFORM POPULATIONS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel 7			8			9		
	Y ($x10^3$/ml)	C No./ml	log	Y ($x10^3$/ml)	C No./ml	log	Y ($x10^3$/ml)	C No./ml	log
0-3 hrs.	1.50	920.0	3.9	8.0	150.0	3.9	3.0	430.0	3.4
1 day	0.50	1470.0	2.1	3.0	4300.0	3.4	1.80	920.0	3.2
2 days	0.60	150.0	2.1	5.0	2500.0	3.4	3.40	73.0	3.5
3	0.10	110.0	2.0	(0.10)	**	(2.0)	(0.10)	**	(2.0)
4	(0.1)	**	(2.0)	(0.10)	**	(2.0)	-	-	-
5	(0.1)	**	(2.0)	(0.10)	**	(2.0)	0.20	**	2.3
6	(0.1)	**	(2.0)	(0.10)	**	(2.0)	0.70	**	2.8
7	(0.1)	**	(2.0)	0.10	**	2.0	6.00	**	3.7
8	-	-	-	0.70	**	2.8	-	-	-
9	2.0	**	3.3	2.50	**	3.3	25.0	**	4.3
10	-	-	-	8.00	**	3.9	30.0	**	4.4
11	16.0	**	4.2	170.0	**	5.2	110.0	**	5.0
13	350.0	**	5.5	120.0	**	5.0	62.0	**	4.7
15	350.0	**	5.5	270.0	**	5.4	40.0	**	4.6
18	85.0	**	4.9	8.0	**	3.9	17.0	**	4.2
21	40.0	**	4.6	20.0	**	4.3	30.0	**	4.4
25	90.0	**	4.9	25.0	**	4.3	4.9	**	3.6
29	50.0	**	4.6	14.0	**	4.1	6.5	**	3.8
33	50.0	**	4.6	25.0	**	4.3	24.0	**	4.3
37	14.0	**	4.1	34.0	**	4.5	14.0	**	4.1
41	10.0	**	4.0	24.0	**	4.3	14.5	**	4.1

- = no determination made
** = less than 10 organisms /ml
(0.001) = less than 100 organisms /ml

Y = yeast populations
C = coliform populations
log = logarithm of yeast counts

TABLE 24

EFFECT OF THE CYSTINE AND TRYPTOPHANE, AND THE CORN STEEP LIQUOR TREATMENTS ON L. plantarum COUNTS IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Third Flask Experiment

Time After Brining	No. of Flask 11		12		13		14	
	No.	log.	No.	log.	No.	log.	No.	log.
0-3 hrs.	128.0	8.1	115.0	8.0	125.0	8.0	112.0	8.0
2 days	2010.0	9.3	1840.0	9.2	2110.0	9.3	1670.0	9.2
6	1450.0	9.1	2250.0	9.3	1220.0	9.0	1200.0	9.0
11	96.0	7.9	93.0	7.9	116.0	8.0	78.0	7.8
17	1.0	6.0	1.1	6.0	8.3	6.9	7.0	6.8
25	0.006	3.7	0.007	3.8	0.48	4.6	0.49	4.6

	15		16		17		18	
	No.	log.	No.	log.	No.	log.	No.	log.
0-3 hrs.	126.0	8.1	128.0	8.1	117.0	8.0	128.0	8.1
2 days	2470.0	9.3	2540.0	9.3	1510.0	9.1	3110.0	9.4
6	1450.0	9.1	930.0	8.9	880.0	8.9	870.0	8.9
11	195.0	8.2	160.0	8.2	193.0	8.2	181.0	8.2
17	12.5	7.0	15.0	7.1	32.0	7.5	37.0	7.5
25	8.7	5.9	8.9	5.9	10.4	7.0	8.1	6.9

No. = counts x 10^6/ml

Flasks 11, 12 = 0.0025% cystine, 0.0025% tryptophane, added
Flasks 13, 14 = 0.0025% cystine, 0.0025% tryptophane, 1.0% sucrose, added
Flasks 15, 16 = 0.821% corn steep liquor, added
Flasks 17, 18 = 0.821% corn steep liquor, 1.0% sucrose, added

TABLE 25

EFFECT OF THE CYSTINE AND TRYPTOPHANE, AND THE CORN STEEP LIQUOR TREATMENTS ON *L. plantarum* POPULATIONS IN BRINES OF LABORATORY CUCUMBER FERMENTATIONS

Fourth Flask Experiment

Time After Brining	No. of Flask 7		8		9		10	
	No.	log	No.	log	No.	log	No.	log
0-3 hrs.	88.0	7.9	90.0	7.9	90.0	7.9	85.0	7.9
1 day	2100.0	9.3	2010.0	9.3	1600.0	9.2	1940.0	9.2
3 days	1940.0	9.2	2200.0	9.3	1800.0	9.2	1700.0	9.2
9	4.0	6.6	13.3	7.1	1.1	6.0	0.2	5.3
35	0.002	3.3	0.005	3.6	0.005	3.6	0.004	3.6
	11		12		13		14	
	No.	log	No.	log	No.	log	No.	log
0-3 hrs.	100.0	8.0	88.0	7.9	100.0	8.0	92.0	7.9
1 day	2860.0	9.4	2650.0	9.4	3000.0	9.4	3180.0	9.5
3 days	3540.0	9.5	2730.0	9.4	2300.0	9.2	2330.0	9.3
9	270.0	8.4	310.0	8.4	78.0	7.8	124.0	8.0
35	0.15	5.1	0.20	5.3	-	-	0.091	4.9

- = no determination made
No. = counts x10^6/ml

Flasks 7, 8 = 0.0025% cystine, 0.0025 tryptophane, added
Flasks 9, 10 = 0.0025% cystine, 0.0025% tryptophane, 1.0% sugar, added
Flasks 11, 12 = 0.821% corn steep liquor, added
Flasks 13, 14 = 0.821% corn steep liquor, 1.0% sugar, added

as yeast numbers, was noted. At one time or another a difference of 1.5 or even more in the log. of counts of the respective organisms occurred. For example, the acid-forming bacteria showed differences of this magnitude on the following days: after 21 days in barrel 7, on the 11th day in barrel 8, and on the 3rd day in barrel 9 (Table 22). Also, comparison between Tables 17 and 23 shows that the log. of yeast counts increased by 1.7 over the control in barrel 9 after 9 days. In all these cases, however, the differences were not consistent and did not last for a considerable period of time.

Under very favorable conditions, results in Tables 24 and 25 indicate no effect as a result of these treatments on L. plantarum counts.

In general, Fig. 5 shows that the acid-forming bacteria increased after brining, reached a peak about the 4th day, maintained high population levels until about the 8th day, then declined gradually for the rest of the examination period. The total bacterial counts closely resembled those of the acid formers. Conversely, yeast counts generally decreased after brining, remained low during the first week of fermentation, then increased to a maximum about the 15th day with little change thereafter (Fig. 6, part B). These results are somewhat different from those of Costilow and Fabian (9), who found that, under commercial conditions, yeasts gradually declined in numbers after reaching a maximum between 10 and 20

days. This gradual decrease in yeast count was not observed in this investigation. Under laboratory conditions, however, Costilow and Fabian (9) reported a fairly high level of yeast population throughout the fermentation period. Thus, the changes in yeast numbers observed in this study under semi-commercial conditions might very well represent the differences in conditions between the fermentations in crocks and commercial tanks.

As to coliform population, an increase in numbers was generally observed on the first day of fermentation (Table 23). The numbers then declined rapidly and very low populations were present on the 4th day. Since large differences in coliform counts existed between individual barrels of similar treatments as previously reported, no significant effect of the addition of cystine and tryptophane alone or in combination with sugar is indicated.

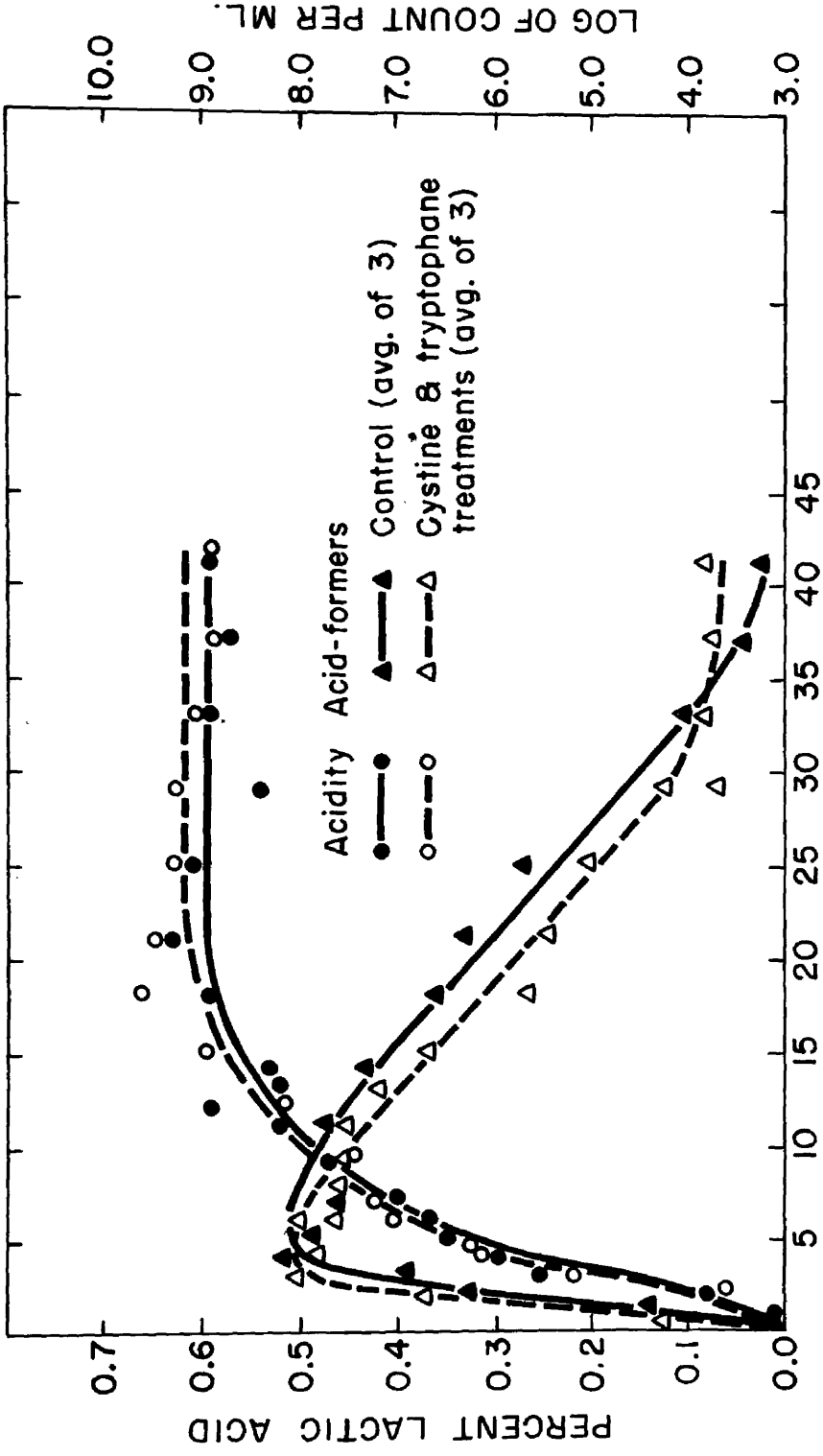

Figure 5. Influence of addition of cystine and tryptophane to brines of semicommercial cucumber fermentations on lactic acid and acid-forming bacteria.

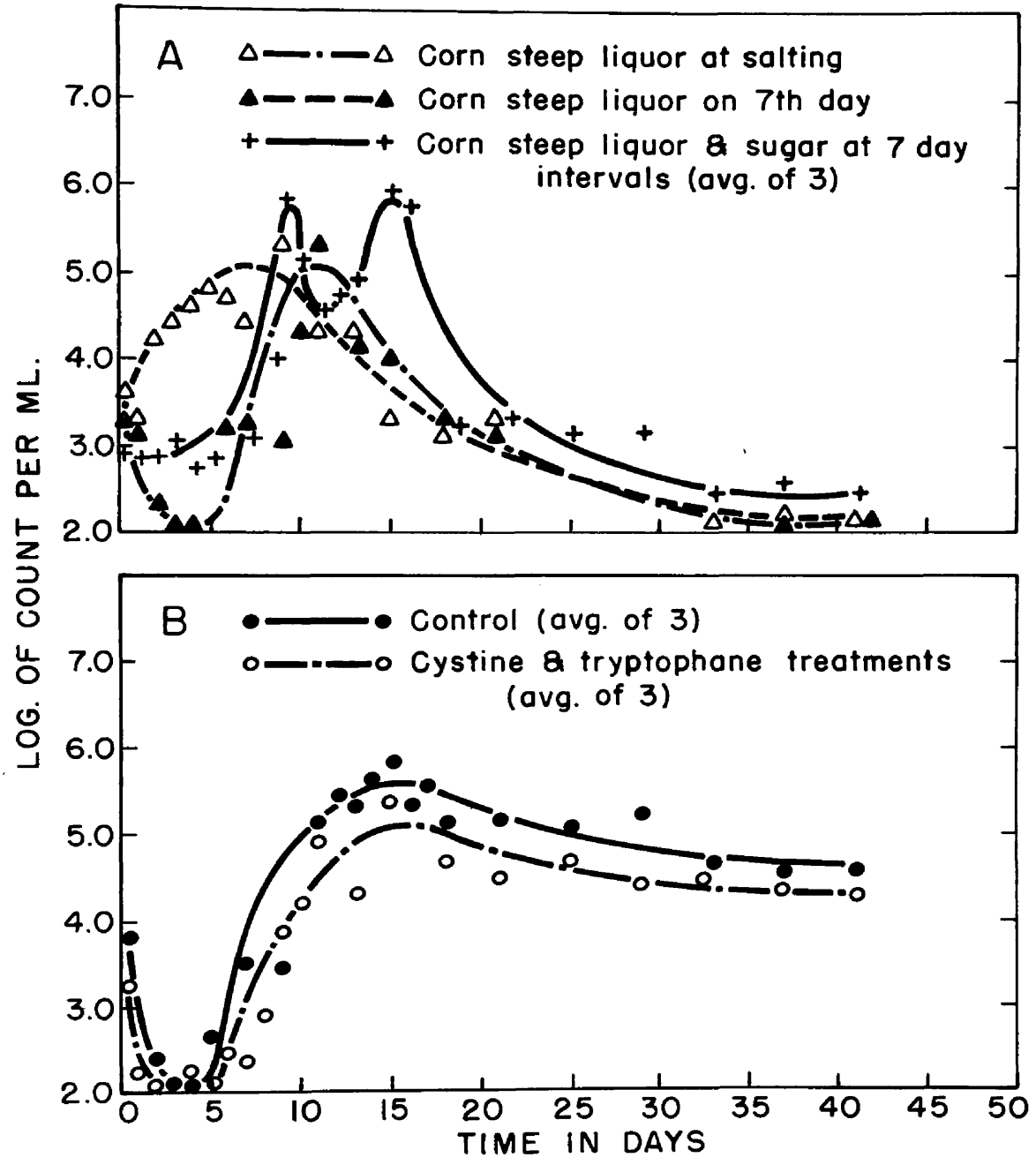

Figure 6. Influence of addition of cystine and tryptophane, and addition of corn steep liquor to brines of semicommercial cucumber fermentations on yeasts.

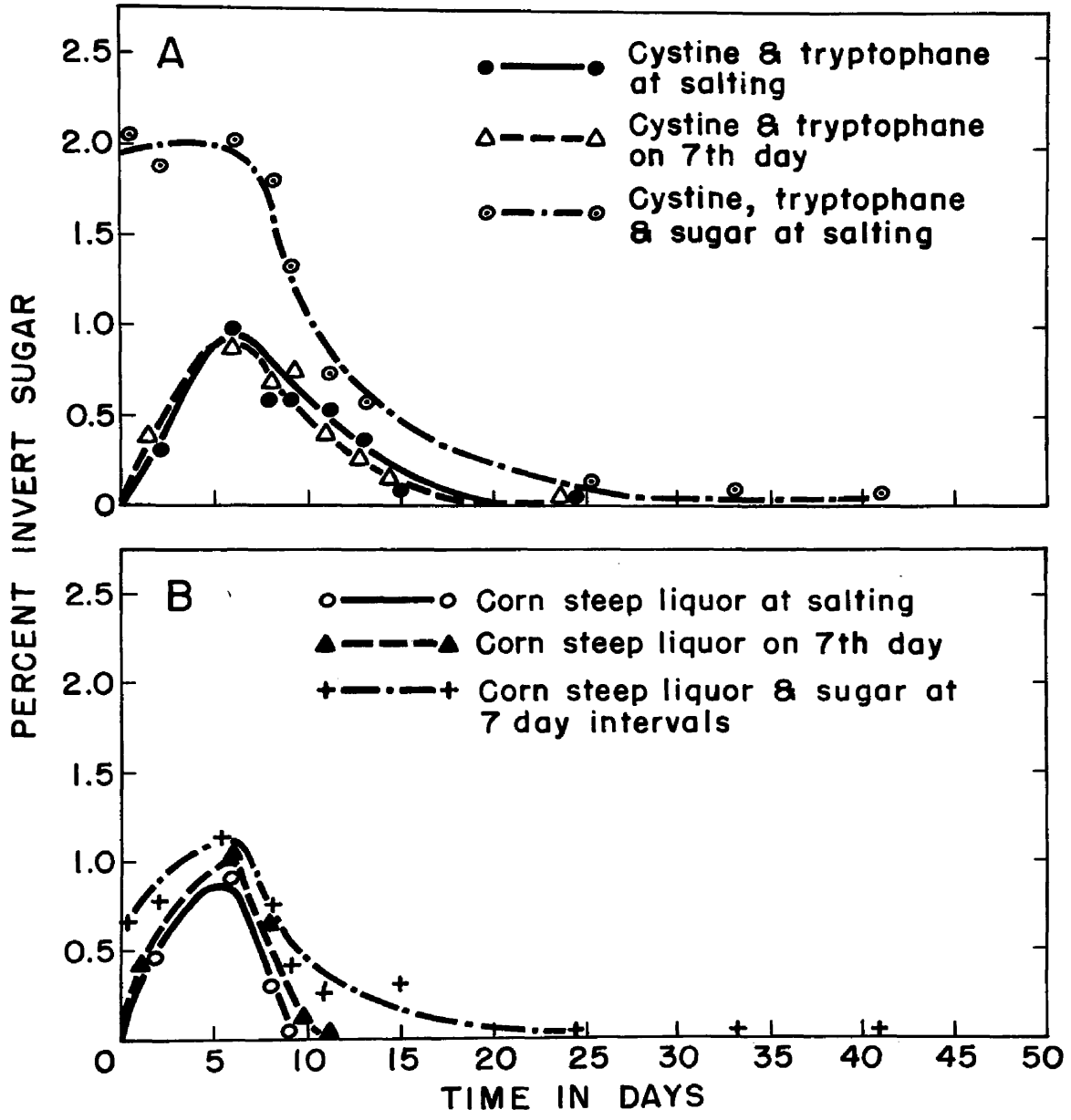

Figure 7. Influence of addition of cystine and tryptophane, and addition of corn steep liquor to brines of semicommercial cucumber fermentations on per cent invert sugar.

III - Influence of Added Corn Steep Liquor on Cucumber Fermentation

Effect of corn steep liquor on lactic acid formation. The effect of addition of corn steep liquor to cucumber salt stock on lactic acid production was investigated along with the studies of sugar, cystine and tryptophane treatments. In 1953, barrels 10 and 11 showed the effect of the corn steep liquor when added at the time of salting and on the 7th day of fermentation respectively. Addition of corn steep liquor in combination with 1.0 per cent sucrose at 7-day intervals was also studied by preparing barrel 12.

Results in Table 1 show that during the first two weeks of fermentation, no significant difference in brine acidity was observed between the corn steep liquor treated fermentations and the control. From the 15th day to the end of the examination period, however, acidity was somewhat higher than that of the control. The maximum increase in acidity was found to be 0.15 per cent. It is true that this difference might occur between similar fermentations within one treatment. Yet, in all corn steep liquor treatments examined, this slight increase of acidity occurred consistently and lasted for a considerable period of time. In 1954, the effect of corn steep liquor was reinvestigated. Barrels 5 and 6 were prepared in the same way as barrel 12 in 1953. Results of this experiment are recorded in Table 2. Here again, the

amount of lactic acid formed during the first two weeks of fermentation in general did not appreciably differ from that of the control. On the other hand, after 18 days of fermentation acidity was higher than in the control by 0.12 per cent. Thereafter, the total titratable acidity remained always higher by at least 0.1 per cent.

It should be mentioned here that the changes in pH levels (Tables 1 and 3), and salt concentrations (Tables 2 and 4) in both 1953 and 1954 experiments were similar in each case to that of the control. No buffering action of corn steep liquor was observed. In addition, the initial pH in brines where corn steep liquor was used, showed no significant difference that might be attributed to the acidity of the corn steep liquor itself.

The average acidity values of all corn steep liquor treatments prepared in 1953 and 1954 are presented in Fig. 8.

The laboratory experiments were then carried out to check the above findings. Table 6 shows that addition of corn steep liquor, alone or in combination with sugar, resulted in a significant rise in acidity on the 11th day and thereafter. For example, on the 25th day acidity increased over the control by 0.12 per cent (for flasks 15, 16); and by 0.19 per cent (for flasks 17, 18). These values are considered significant since they exceeded by far the variations found among replicate determinations. Fig. 4, part C, represents

the average acidity values obtained when corn steep liquor was used in the 3rd laboratory experiment.

When the flask experiment was repeated (Table 7), the increase in brine acidity was even greater and more pronounced throughout the whole examination period.

One can only conclude, then, that the increase in brine acidity observed under semicommercial conditions is significant.

Results obtained with the reducing sugars content in fermenting brines under laboratory conditions indicate that when corn steep liquor was added, L. plantarum was able to utilize more of the naturally occurring sugars in the cucumbers as compared to the control (Table 8). Thus the amount of invert sugar utilized in flasks 11 and 12 was 1.279 per cent as compared to 0.902 per cent (avg. of flasks 1, 2) in the control. The combined treatment of corn steep liquor and 1.0 per cent sucrose showed almost the same effect. Therefore, utilization of reducing sugars remained the same as when corn steep liquor only was used. Apparently, then, the added sugar was not utilized by L. plantarum.

In 1953 and under semicommercial conditions, the reducing sugars of brines of all three fermentations examined (barrels 10, 11, 12), rapidly increased after brining and reached a maximum similar to that of the control approximately after 6 days (Fig. 7, part A). Thereafter, the reducing sugars rapidly decreased. For example, in barrel 10 no invert sugar was

detected on the 9th day as compared to 0.7 per cent in the control (Table 9). A similar correlation was observed in barrel 11 on the 11th day. When corn steep liquor was applied in combination with 1.0 per cent sucrose, the reducing sugars decreased rapidly even after adding the second portion of sugar. However, the acidity remained the same in all corn steep liquor treatments. Therefore, the added sugar was probably utilized mainly by yeasts. The increase in acidity over that of the control when corn steep liquor was added is mostly due to greater utilization of the carbohydrates in the cucumbers by the acid-forming bacteria.

Effect of corn steep liquor on total and acid-forming bacteria. The populations of total and acid-forming bacteria observed during the fermentation of cucumber salt stock which received the corn steep liquor treatments in 1953 and 1954 are given in Table 26.

A variation of more than 1.5 in the log. of the bacterial numbers was considered significant here as previously. The average populations of acid-forming bacteria in five corn steep liquor barrels and three control barrels prepared in 1953 and 1954 is illustrated in Fig. 8. It is evident from this figure that there was no significant difference during the first two weeks of fermentation between the corn steep liquor treatments and the control. In general, the populations rapidly increased after brining and reached a maximum

TABLE 26

EFFECT OF CORN STEEP LIQUOR TREATMENTS ON TOTAL AND ACID-FORMING BACTERIAL POPULATIONS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

Time After Brining	No. of Barrel									
	1953 Fermentations						1954 Fermentations			
	10		11		12		5		6	
	T	A	T	A	T	A	T	A	T	A
	($x10^6$/ml)		($x10^6$/ml)		($x10^6$/ml)		($x10^6$/ml)		($x10^6$/ml)	
0-3 hrs.	0.31	(0.001)	0.84	(0.001)	0.57	(0.001)	(0.001)	(0.001)	(0.001)	(0.001)
1 day	0.35	0.003	0.38	0.12	1.20	0.06	-	-	-	-
2 days	0.80	0.80	13.0	9.0	3.0	3.0	7.2	6.2	21.5	15.0
3	165.0	140.0	110.0	84.0	100.0	75.0	86.0	95.0	108.0	135.0
4	185.0	180.0	110.0	70.0	106.0	185.0	228.0	266.0	100.0	108.0
5	257.0	320.0	100.0	77.0	187.0	215.0	176.0	141.0	138.0	127.0
6	300.0	350.0	55.0	60.0	50.0	60.0	-	-	-	-
7	170.0	175.0	25.0	20.0	57.0	75.0	117.0	165.0	121.0	113.0
8	-	-	17.0	10.0	50.0	80.0	-	-	-	-
9	75.0	110.0	10.0	10.0	55.0	70.0	92.0	95.0	390	310
10	-	-	7.0	7.0	50.0	65.0	-	-	-	-
11	50.0	60.0	7.2	8.5	23.0	25.0	-	-	-	-
12	-	-	-	-	-	-	26.0	18.0	14.7	12.2
13	24.0	21.0	2.5	3.5	21.0	20.0	-	-	-	-
14	-	-	-	-	-	-	19.0	20.9	17.0	10.4
15	16.0	15.0	1.6	3.0	21.5	26.5	-	-	-	-
16	-	-	-	-	11.0	12.5	14.0	15.0	21.0	8.3
17	-	-	-	-	10.0	6.5	-	-	-	-
18	2.00	2.5	1.0	1.4	10.0	16.0	16.0	18.0	27.0	17.8
21	0.21	2.2	1.40	1.3	15.0	10.0	6.5	9.0	8.0	2.0
25	0.50	3.2	1.40	1.5	2.3	1.40	8.0	8.0	3.0	3.4
29	0.10	0.18	0.20	0.15	0.14	0.17	10.0	3.0	2.0	1.2
33	0.05	0.10	-	0.20	0.13	0.17	3.0	1.5	1.5	1.2
37	0.12	0.12	0.10	0.12	0.10	0.45	0.7	0.5	0.7	0.8
41	0.05	0.05	0.10	0.06	0.12	0.10	0.5	0.5	0.7	0.7

- = no determination made
A = acid-producing bacteria counts
T = total bacteria counts

TABLE 27

DIFFERENCE IN LOG. OF ACID-FORMING BACTERIA COUNTS WHEN DIFFERENT CORN STEEP LIQUOR TREATMENTS WERE APPLIED TO SEMICOMMERCIAL CUCUMBER FERMENTATIONS

Time After Brining	No. of Barrel											
	1953 Fermentations							1954 Fermentations				
	1	10	D_{1-10}	11	D_{1-11}	12	D_{1-12}	1	5	D_{1-5}	6	D_{1-6}
0-3 hrs.	(3.0)	(3.0)	0	(3.0)	0	(3.0)	0	(3.0)	(3.0)	0	3.0	0
1 day	4.4	3.4	1.0	5.0	0.6	4.7	0.3	-	-	-	-	-
2 days	6.4	5.9	0.5	6.9	0.5	6.4	0	6.3	6.7	0.4	7.1	0.8
3	6.8	8.1	1.3	7.9	1.1	7.8	1.0	6.9	7.9	1.0	8.1	1.2
4	7.8	8.2	0.4	7.8	0	8.2	0.4	8.3	8.4	0.1	8.0	0.3
5	7.2	8.5	1.3	7.8	0.6	8.3	1.1	8.1	8.1	0	8.0	0.1
6	7.3	8.5	1.2	7.7	0.4	7.7	0.4	-	-	-	-	-
7	7.0	8.2	1.2	7.3	0.3	7.8	0.8	7.7	8.2	0.5	8.0	0.3
8	-	-	-	7.0	-	7.9	-	-	-	-	-	-
9	6.6	8.0	1.4	7.0	0.4	7.8	1.2	7.5	7.9	0.4	7.4	0.1
10	-	-	-	6.8	-	7.8	-	-	-	-	-	-
11	6.3	7.7	1.4	6.9	0.6	7.3	1.0	-	-	-	-	-
12	-	-	-	-	-	-	-	7.0	7.2	0.2	7.0	0
13	6.0	7.3	1.3	6.5	0.5	7.3	1.3	-	-	-	-	-
14	-	-	-	-	-	-	-	6.9	7.3	0.4	7.0	0.1
15	5.8	7.1	1.3	6.4	0.6	7.4	1.6	-	-	-	-	-
16	-	-	-	-	-	7.0	-	-	7.1	-	6.9	-
17	-	-	-	-	-	6.8	-	-	-	-	-	-
18	5.5	6.3	0.8	6.1	0.6	7.2	1.7	6.7	7.2	0.5	7.2	0.5
21	5.5	6.3	1.2	6.1	0.6	7.0	1.5	6.3	6.9	0.6	6.3	0
25	4.6	6.5	1.1	6.1	1.5	6.1	1.5	5.6	6.9	1.3	6.5	0.9
29	3.0	5.2	2.2	5.1	2.1	5.1	2.1	4.3	6.4	2.1	6.0	1.7
33	3.3	5.0	1.7	5.3	2.0	5.1	1.8	4.3	6.1	1.7	6.0	1.7
37	(3.0)	5.0	2.0	5.0	2.0	5.1	2.1	3.6	5.6	2.0	5.9	2.3
41	3.2	4.6	1.4	4.3	1.5	5.0	1.8	3.4	5.6	2.2	5.8	0.4

- = no determination made

D = difference in log. of acid-forming bacterial counts between the control and the specific treatment

of about 100 x 10^6/ml in from 3 to 5 days. Table 26 also shows that the time of adding corn steep liquor during fermentation was not an influencing factor. In all corn steep liquor treatments, however, it was noted that the numbers of acid-formers were higher than those of the control on the 29th day and thereafter. Table 27 shows that except for barrels 10 and 11 after 41 days, the log. of counts of these organisms was higher than that of the control by at least 1.7 (about 50 times greater). Even in barrels 10 and 11, the numbers of acid-forming bacteria were about 32 times greater than those of the control after 41 days. It is true that in these two examinations the observed difference did not exceed the limit of normal variation that might be found between similar treatments. However, some significance should be given to it since the largest difference in the log. of acid-forming bacteria between duplicate determinations prepared in one season was always smaller than this, i.e., 1.2 (Tables 12, 13 and 27).

The over-all total bacterial changes during fermentation resembled those of the acid formers. In general the population increased after brining and reached a peak in from 3 to 6 days. The numbers then declined rapidly to the end of the examination period. Between 29 and 41 days, however, the total bacterial numbers maintained a higher level than that of the control. A corresponding increase in acid-forming bacteria has been shown before.

Effect of corn steep liquor on yeasts and coliforms.

Results of the studies on population changes of yeasts during fermentation of 3 barrels in 1953 and 2 barrels in 1954, are presented in Table 28 and Fig. 6, part A. It is evident from these results that in all corn steep liquor treatments, the yeast population increased to a maximum during the second week of fermentation. A rapid decrease in numbers then occurred until the level was generally lower than that of the control on the 18th day. During the rest of the fermentation period that followed, the yeast numbers remained lower than those of the control. In only two instances during this period, however, namely in barrels 11 and 12 after 29 days, was there no significant difference between the corn steep liquor treatments and the control (Table 29). As a matter of fact, yeasts were even slightly lower in numbers than in the control. This is of doubtful significance, however, and is most likely due to experimental error.

There is some evidence that combining sugar with corn steep liquor might have some effect on the yeast population. For example, yeast numbers in barrel 12 increased from 2.5 x 10^3/ml on the 7th day to 130 x 10^3/ml on the 9th day. (Table 28). The time of adding the corn steep liquor did not greatly influence yeast population. It was noted only that yeast numbers were higher than in the control between the first 3 to 5 days of fermentation when corn steep liquor was added at the time of salting. No such difference, however, was observed when

corn steep liquor was added in small portions throughout the fermentation. This was probably due to the fact that considerably smaller amounts of corn steep liquor and sugar were added at the time of salting.

The coliform populations varied considerably from one barrel to another. There is no indication that addition of corn steep liquor, alone or in combination with sugar, had any effect on the numbers of coliforms present during fermentation. In general coliforms disappeared after 6 days. Apparently unfavorable conditions in the brine inhibited them. Similar to the treatments with sugar, cystine and tryptophane, the coliforms played no significant part when corn steep liquor was applied.

TABLE 28

EFFECT OF CORN STEEP LIQUOR TREATMENTS ON YEASTS AND COLIFORM POPULATIONS IN BRINES OF SEMICOMMERCIAL CUCUMBER FERMENTATIONS

Time After Brining	No. of Barrel									
	1953 Fermentations						1954 Fermentations			
	10		11		12		5		6	
	Y x10^3/ml	C No./ml	Y x10^3/ml	C No./ml	Y x10^3/ml	C No./ml	Y x10^3/ml	C No./ml	Y x10^3/ml	C No./ml
0-3 hrs.	4.0	740	2.1	250	0.62	43	1.0	430	1.6	920
1 day	2.1	430	1.3	250	0.84	74	-	- -	-	- -
2 days	17.0	24	0.24	430	0.20	430	1.7	4300	1.0	9200
3	30.0	220	0.10	**	0.10	73	1.9	91	1.0	36
4	42.0	92	0.10	**	1.0	**	0.70	24	0.2	**
5	65.0	43	0.10	**	0.95	**	0.80	**	0.5	**
6	59.0	25	1.6	**	26.0	**	-	-	-	-
7	31.0	**	1.8	**	2.5	**	1.6	**	1.2	**
8	-	-	0.1	**	10.0	**	-	-	-	-
9	230.0	**	1.0	**	130.0	**	960.0	**	1000.0	**
10	-	-	21.0	**	100.0	**	-	-	-	-
11	25.0	**	250.0	**	38.0	**	-	-	-	-
12	-	-	-	-	-	-	18.8	**	88.0	**
13	24.0	**	14.0	**	81.0	**	-	-	-	-
14	-	-	-	-	-	-	90.0	**	82.0	**
15	2.8	**	10.0	**	800.0	**	-	-	-	-
16	-	-	-	-	700.0	**	600.0	**	890.0	**
17	-	-	-	-	32.0	**	-	-	-	-
18	1.5	**	2.3	**	1.0	**	1.22	**	2.9	**
21	2.1	**	1.5	**	-	-	2.30	**	1.9	**
25	1.0	**	1.0	**	1.0	**	1.70	**	1.8	**
29	-	-	1.0	**	1.0	**	1.39	**	1.6	**
33	0.10	**	-	-	0.15	**	0.45	**	0.33	**
37	0.16	**	0.10	**	0.13	**	0.65	**	0.20	**
41	0.18	**	0.13	**	0.18	**	-	-	0.38	**

- = no determination made
** = less than 10 organisms /ml
C = coliform counts
Y = yeast counts

TABLE 29

DIFFERENCE IN THE LOG. OF YEAST COUNTS WHEN DIFFERENT CORN STEEP LIQUOR TREATMENTS WERE APPLIED TO SEMICOMMERCIAL CUCUMBER FERMENTATIONS

Time After Brining	No. of Barrel											
	1953 Fermentations							1954 Fermentations				
	1	10	D_{1-10}	11	D_{1-11}	12	D_{1-12}	1	5	D_{1-5}	6	D_{1-6}
0-3 hrs.	3.4	3.6	0.2	3.3	0.1	2.7	0.7	3.7	3.0	0.7	3.2	0.5
1 day	3.1	3.3	0.2	3.1	0	2.9	0.2	-	-	-	-	-
2 days	2.8	4.2	1.4	2.3	0.5	2.3	0.5	(2.0)	3.2	1.2	3.0	1.0
3	2.3	4.4	2.1	2.0	0.3	2.0	0.3	(2.0)	3.2	1.2	3.0	1.0
4	(2.0)	4.6	2.6	2.0	0	3.0	1.0	(2.0)	2.8	0.8	2.3	0.3
5	2.0	4.8	2.8	2.0	0	2.9	0.9	2.6	2.9	0.3	2.6	0
6	2.0	4.7	2.7	3.2	1.2	4.4	2.4	-	-	-	-	-
7	3.0	4.4	1.4	3.2	0.2	3.3	0.3	3.6	3.2	0.4	3.0	0.6
8	2.0	-	-	2.0	0	4.0	2.0	-	-	-	-	-
9	2.6	5.3	2.7	3.0	0.4	5.1	2.5	4.5	5.9	1.4	6.0	1.5
10	3.0	-	-	4.3	1.3	5.0	2.0	-	-	-	-	-
11	5.1	4.3	0.2	5.3	0.2	4.5	0.6	-	-	-	-	-
12	-	-	-	-	-	-	-	5.2	4.2	1.0	4.9	0.3
13	5.3	4.3	1.0	4.1	1.2	4.9	0.4	-	-	-	-	-
14	-	-	-	-	-	-	-	5.6	4.9	0.7	4.9	0.7
15	5.8	3.4	2.4	4.0	1.8	5.9	0.1	-	-	-	-	-
16	5.3	-	-	-	-	5.8	0.5	-	5.1	-	5.9	-
17	5.5	-	-	-	-	4.5	1.0	-	-	-	-	-
18	5.3	3.1	2.2	3.3	2.0	3.0	2.3	5.0	3.0	2.0	3.4	1.6
21	5.0	3.3	1.7	3.1	1.9	-	-	5.3	3.3	2.0	3.2	2.1
25	5.0	3.0	2.0	3.0	2.0	3.0	2.0	-	3.2	-	3.2	-
29	3.8	-	-	3.0	0.8	3.0	0.8	5.6	3.1	2.5	3.2	2.4
33	4.2	2.0	2.2	-	-	2.1	2.1	4.7	2.6	2.1	2.5	2.2
37	4.3	2.2	2.1	2.0	2.3	2.1	2.2	4.7	2.8	1.9	1.3	2.4
41	4.3	2.0	2.3	2.1	2.2	2.2	2.1	4.2	-	-	2.5	1.7

- = no determination made
D = difference in the log. of yeast count between the specific treatment and the control.

(2.0) = less than 100 organisms/ml. The log. of count was considered equal to 2.0 when comparative differences were studied.

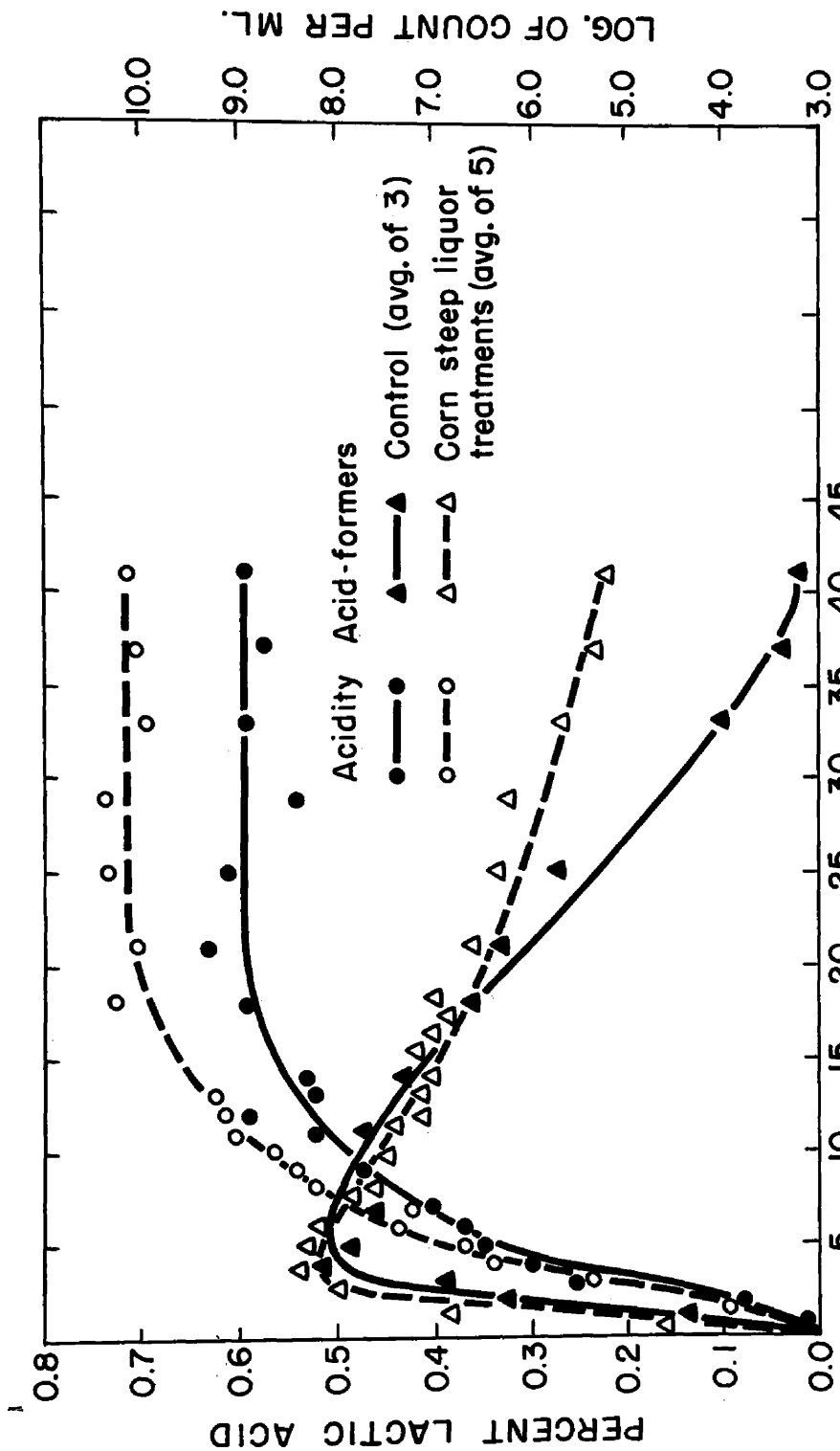

Figure 8. Influence of addition of corn steep liquor to brines of semicommercial cucumber fermentations on lactic acid and acid-forming bacteria.

IV - Vitamins and Amino Acids in Semicommercial Fermentations

It is well known that a prompt and appreciable acid production in fermenting brines is desirable. This decreases the possibility of undesirable types of microorganisms utilizing a large quantity of the available sugar in the brine which would result in a low final acidity. Since L. plantarum is considered by many workers as one of the major groups of organisms responsible for acid formation, the presence of plentiful amounts of the essential vitamins and amino acids is important. Therefore, it was interesting in this study to investigate the changes that occur in these vitamins and amino acids and which might be a result of the addition of sugar, cystine and tryptophane, or corn steep liquor. The complete results of the different fermentations prepared in 1953 are shown in Tables 31 through 33. With the exception of tryptophane, the brine contents of the vitamins: biotin, niacin and pantothenic acid; and the amino acids: leucine, isoleucine, cystine, glutamic acid, and valine, in general gradually increased after brining. This was due to leaching of the different nutrients from the cucumbers into the brine. After reaching a maximum, rather high concentration levels were maintained until the end of the examination period. Thus all three vitamins and five amino acids appear not to be limiting factors for the growth of acid-forming bacteria. Significant amounts are probably present to satisfy the

growth requirements of L. plantarum. It was also observed that the general trend of changes in the levels of these vitamins and amino acids was the same for all the different treatments when applied to the different cucumber barrels. Addition of different amounts of sugar at different times throughout the fermentation, showed no significant difference that can be attributed to the specific treatment. High concentrations were generally observed when corn steep liquor was applied, with no effect as a result of combining it with sugar. Brines in barrels 7, 8 and 9 were richer in cystine content as compared to the control (Table 31). Undoubtedly, this was a result of adding 0.0025 per cent of cystine to each of these barrels.

From the standpoint of tryptophane, the change in concentrations, especially at the end of the examination period, was quite different. In general, tryptophane increased gradually after salting as a result of withdrawing nutrients from the cucumber by osmosis. Except for the corn steep liquor treatments, the tryptophane concentrations after reaching a maximum, markedly decreased about the 11th day and continued to decrease thereafter. For example, a rapid decline in the tryptophane concentrations occurred between the 11th and 25th day in the control barrel as well as in the fermentations where sugar was applied at the time of salting (Fig. 9). When sugar was added on the 7th day, or at 7-day intervals, a drop in

tryptophane levels occurred even earlier, namely on the 9th day. Comparison between Tables 28 and 30 show that tryptophane decreased when yeast activity was greatest. Thus, in barrel 3 the tryptophane concentration decreased from 18 µg/ml on the 6th day to 11.5 µg/ml on the 9th day. During the same period, yeast population increased from less than 100 organisms per ml to 6900 x 10^3/ml. Similarly, in barrel 5, tryptophane decreased from 19.7 µg/ml to 7.4 µg/ml, while yeast numbers increased from less than 100 cells per ml to 38000 x 10^3/ml. This indicates a rapid utilization of tryptophane by yeasts. A similar finding was reported by Costilow and Fabian (9), in cucumber salt stock under laboratory and commercial conditions.

A good illustration of the tryptophane decline occurred in barrel 8 (Fig. 10). Following the addition of tryptophane in combination with cystine on the 7th day, the tryptophane concentrations increased from 15.6 µg/ml on the 6th day to 44.6 µg/ml on the 9th day. However, a rapid drop occurred and the tryptophane levels decreased to 15.1 µg/ml on the 25th day. In general, the tryptophane concentrations in barrels 7, 8 and 9, were somewhat higher at the end of the examination period than in the control. This indicates that the added tryptophane was not completely utilized by the brine microorganisms. Since no greater amounts of acid formation occurred as a result of these treatments, it can be concluded that sufficient tryptophane was present in the cucumbers for the growth of the acid-forming population.

When corn steep liquor was added, tryptophane concentrations generally increased after brining, and reached a maximum similar to that of the control (Fig. 11). With the exception of the combined treatment of corn steep liquor and sugar, the tryptophane levels in all other corn steep liquor treatments did not show a rapid decline on the 11th day as the control. A slight decrease was observed only at the end of the examination period. Fig. 11 is a good illustration of the difference in the tryptophane levels during fermentation of the corn steep liquor treatments as compared to the control. Evidently, considerably smaller amounts of tryptophane were utilized by the brine microorganisms. This correlates with the fact that lower yeast populations were found after 18 days when corn steep liquor was applied. On the other hand, the acid-forming bacteria showed a marked increase in numbers over the control. It has been previously reported that more and faster utilization of reducing sugar occurred when corn steep liquor was applied under laboratory and semicommercial conditions. Apparently, corn steep liquor enhanced the utilization of the available sugar in the brine resulting in the production of greater amounts of acid.

When corn steep liquor was applied in combination with sugar, the decline in the tryptophane levels was greater at the end of the examination period than in the case where

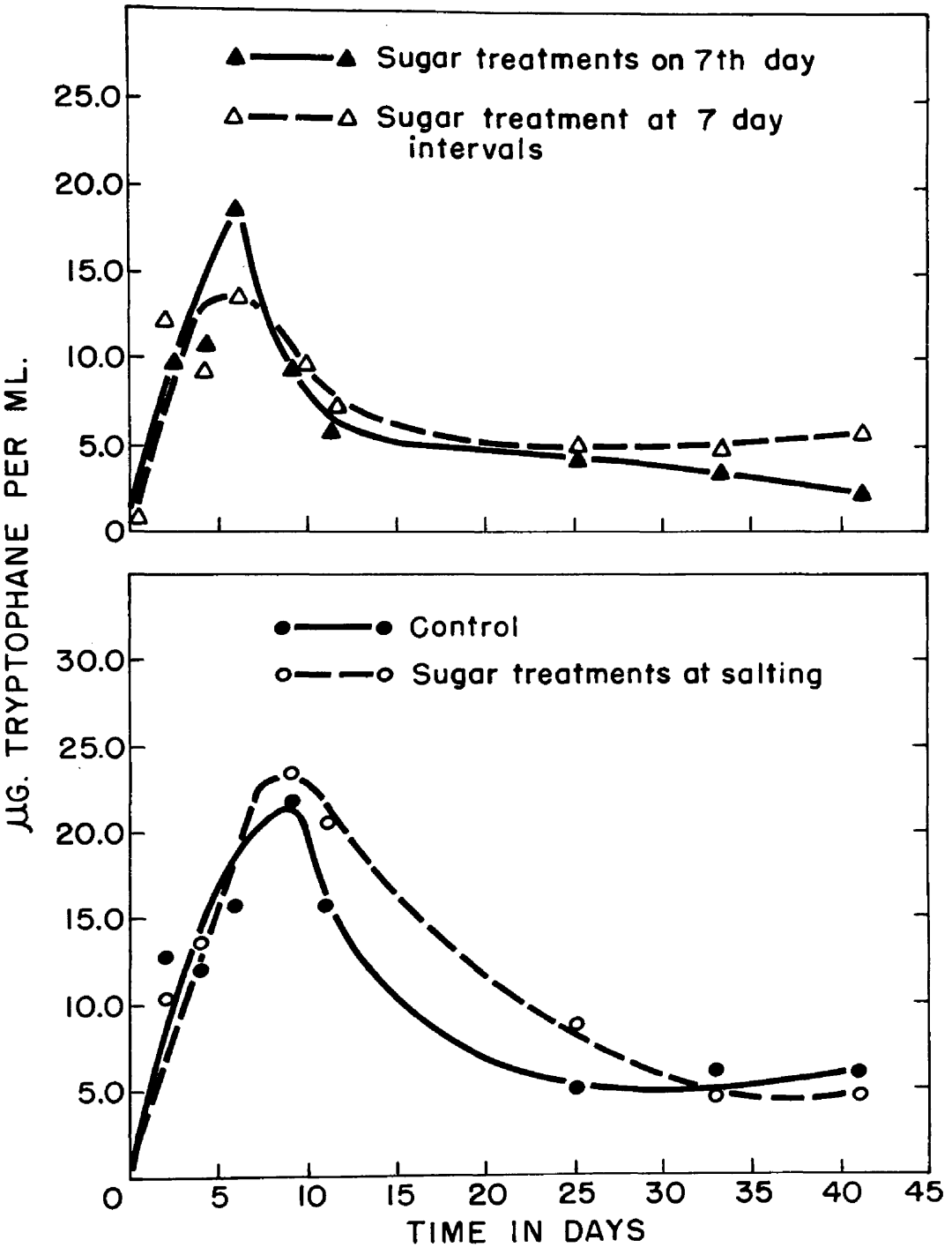

Figure 9. Influence of addition of sugar to brines of semicommercial cucumber fermentations on tryptophane.

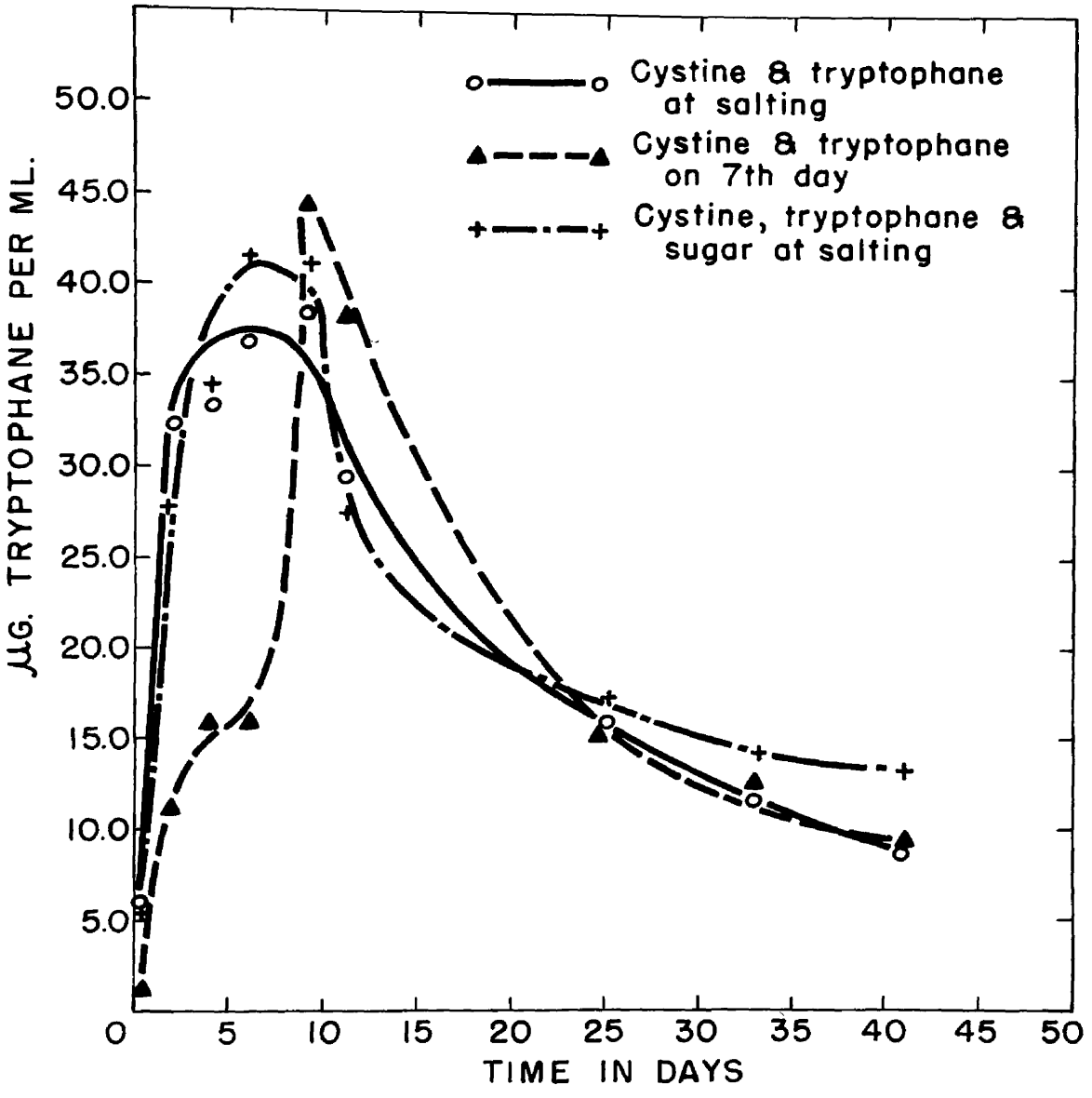

Figure 10. Influence of addition of cystine and tryptophane to brines of semi-commercial cucumber fermentations on tryptophane.

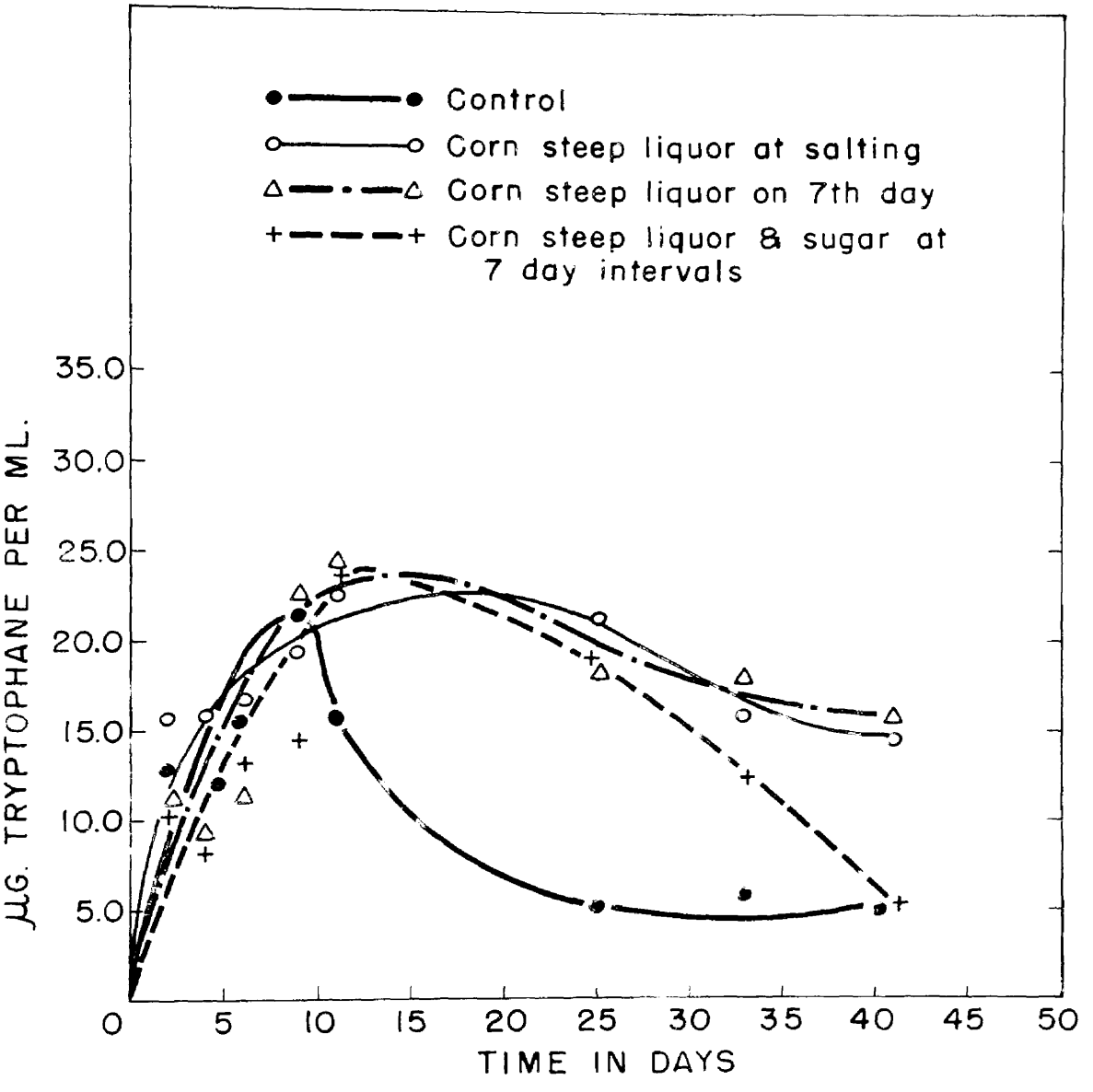

Figure 11. Influence of addition of corn steep liquor to brines of semicommercial cucumber fermentations on tryptophane.

TABLE 30

A COMPARISON OF THE TRYPTOPHANE, CYSTINE AND VALINE CONCENTRATIONS IN BRINES OF DIFFERENT SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	(µg/ml) No. of Barrel											
	1	2	3	4	5	6	7	8	9	10	11	12
Tryptophane												
0-3 hrs.	0.89	0.53	0.68	0.42	0.47	0.42	5.80	0.38	5.01	0.83	0.36	1.28
2 days	12.60	12.80	11.00	7.80	9.50	12.20	32.10	11.00	28.44	15.55	11.11	10.03
4	11.90	16.55	12.10	10.20	10.15	9.35	33.25	15.90	34.35	15.45	9.35	8.05
6	15.80	15.65	18.00	11.40	19.70	13.60	36.51	15.60	41.50	16.70	11.16	13.36
9	21.70	24.50	11.50	22.50	7.40	9.40	37.32	44.60	41.00	19.50	22.74	14.40
11	15.90	20.80	6.30	20.31	5.55	7.05	29.00	38.11	27.00	22.53	24.45	23.90
25	5.10	10.90	7.60	6.52	1.50	4.30	15.87	15.10	17.20	21.30	18.20	17.40
33	6.10	5.45	5.00	4.33	2.40	4.70	11.55	12.40	14.21	16.30	18.13	12.37
41	6.00	5.80	3.00	4.15	1.50	5.80	8.65	9.10	13.00	14.40	15.85	5.10
Cystine												
0-3 hrs.	*	*	*	*	*	*	3.25	*	3.06	9.75	*	19.00
2 days	8.11	6.53	4.40	4.70	8.61	8.38	33.88	8.43	31.99	24.88	7.99	29.10
9	23.20	25.90	23.25	26.47	23.48	23.70	59.40	54.44	66.89	40.25	47.33	42.07
25	21.44	24.60	29.21	25.66	28.40	26.22	62.43	54.00	65.25	41.00	38.40	42.19
41	19.30	18.22	29.26	27.10	27.17	26.40	54.90	54.43	62.31	38.40	37.20	37.11
Valine												
0-3 hrs.	**	**	**	**	**	**	**	**	**	58.25	**	**
2 days	22.11	38.21	19.35	29.85	28.85	18.85	12.00	18.00	17.35	36.50	16.75	24.70
9 days	105.05	94.70	91.35	91.25	99.15	107.50	92.30	91.25	87.75	144.75	140.65	109.35
25	90.00	102.95	107.70	96.40	104.05	110.85	103.75	119.15	117.00	159.45	152.40	157.95
41	133.31	128.37	119.37	113.30	123.95	124.35	133.30	133.30	128.75	157.90	184.55	149.92

* = less than 0.5 µg/ml of cystine

** = less than 2.5 µg/ml of valine

TABLE 31

A COMPARISON OF LEUCINE, ISOLEUCINE AND GLUTAMIC ACID CONCENTRATIONS IN BRINES OF DIFFERENT SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	(µg/ml) No. of Barrel											
	1	2	3	4	5	6	7	8	9	10	11	12
L-leucine												
0-3 hrs.	*	*	*	*	*	*	*	*	*	191.50	*	72.50
2 days	267.50	235.81	200.00	176.66	206.60	229.18	170.80	194.17	208.30	405.00	210.80	267.50
9	245.00	279.12	281.64	242.55	254.11	243.30	245.00	272.50	245.00	428.30	410.00	323.36
25	255.00	281.65	298.30	260.00	245.09	256.60	256.60	325.00	281.60	412.52	386.60	416.62
41	292.10	320.00	285.00	255.00	252.59	272.50	282.53	325.00	271.00	420.00	395.50	420.00
L-isoleucine												
0-3 hrs.	**	**	**	**	**	**	**	**	**	54.00	**	19.00
2days	71.50	62.00	50.27	49.25	49.00	59.50	44.50	50.57	55.20	108.70	58.21	57.00
9	151.81	154.17	156.20	134.50	136.20	149.50	135.44	133.79	135.82	197.00	183.70	142.99
25	145.40	162.59	167.90	146.69	146.55	139.10	153.70	178.35	156.60	228.75	202.50	200.40
41	159.66	163.00	160.65	147.36	142.80	143.88	148.00	167.60	153.40	208.63	192.37	196.10
Glutamic acid												
0-3 hrs.	***	***	***	***	***	***	***	***	***	37.56	***	18.50
2 days	92.20	87.01	77.00	53.20	83.50	44.00	33.25	33.50	43.20	108.20	82.20	55.00
9	166.65	158.26	148.01	148.51	156.50	164.50	146.70	148.00	131.50	173.70	170.70	136.50
25	137.50	142.50	143.00	145.56	145.00	139.00	132.50	139.50	131.70	155.50	151.20	134.22
41	137.50	142.10	152.10	152.00	139.50	132.50	139.95	140.11	129.70	151.20	150.70	146.00

* = less than 5.0 µg/ml of leucine
** = less than 2.5 µg/ml of isoleucine
*** = less than 5.0 µg/ml of glutamic acid

TABLE 32

A COMPARISON OF BIOTIN, NIACIN AND PANTOTHENIC ACID CONCENTRATIONS IN BRINES OF DIFFERENT SEMICOMMERCIAL CUCUMBER FERMENTATIONS

1953 Fermentations

Time After Brining	No. of Barrel											
	1	2	3	4	5	6	7	8	9	10	11	12
Biotin(mμg/ml)												
0-3 hrs.	*	*	*	*	*	*	*	*	*	1.25	*	2.25
2 days	6.62	2.97	2.40	3.71	3.82	3.32	3.52	3.70	2.54	4.58	3.48	3.50
9	7.72	8.40	5.62	7.23	7.14	8.22	5.89	5.40	5.55	8.58	8.50	5.75
25	6.90	6.82	5.70	7.57	8.19	6.91	8.89	6.90	6.72	11.28	12.50	7.30
41	5.15	5.32	4.95	6.47	6.32	6.35	7.95	5.02	5.72	10.12	9.55	8.47
Niacin(μg/ml)												
0-3 hrs.	0.075	**	**	**	**	**	**	**	**	0.542	**	0.202
2 days	0.518	0.646	0.519	0.685	0.413	0.584	0.830	0.532	0.596	1.385	0.542	0.500
9	1.375	1.455	1.600	1.556	1.320	1.616	1.430	1.572	1.566	1.445	1.544	1.193
25	1.166	1.140	1.484	1.438	1.294	1.514	1.373	1.482	1.450	1.450	1.485	1.495
41	1.360	1.281	1.488	1.370	1.120	1.581	1.387	1.568	1.481	1.390	1.462	1.480
Pantothenic acid(μg/ml)												
0-3 hrs.	***	***	***	***	***	***	***	***	***	***	***	***
2 days	1.690	1.850	1.055	1.437	1.482	1.265	1.077	1.002	1.125	2.482	1.875	1.482
9	1.954	1.532	1.192	1.450	1.487	1.375	1.925	1.132	1.427	2.470	2.187	1.737
25	1.620	1.400	1.177	1.457	1.342	1.395	1.807	1.347	1.277	2.512	2.430	2.345
41	1.425	1.425	1.230	1.490	1.300	1.375	1.695	1.330	1.320	2.445	2.400	2.490

* = less than 0.025 mμg/ml of biotin
** = less than 0.05 μg/ml of niacin
*** = less than 0.02 μg/ml of pantothenic acid

corn steep liquor was applied alone. This was probably a result of increased yeast activity. However, since a similar increase in acidity occurred as in the other corn steep liquor treatments, it seems likely that the tryptophane concentrations were not a limiting factor for the growth of acid formers. Sufficient tryptophane was probably present to cover the needs of the acid-forming bacteria.

DISCUSSION

Cucumber fermentation is a complex process. It depends on many factors such as salt concentration, climatic conditions, the variety and size of cucumbers, and several other factors. Addition of sugar to cucumber salt stock with the object of promoting faster and greater production of lactic acid has been studied by many investigators. Discrepancies are found in the literature, however, as to the beneficial effects of this treatment. In this investigation, addition of 0.5 or 1.0 per cent sucrose did not accelerate or increase acid formation. These results agree with those of other studies (33, 55). The increased acid formation reported by many workers probably was due to the variations that normally occur among individual cucumber fermentations.

The populations of total and acid-forming bacteria were not affected by the different sugar treatments. It has been mentioned before that the older literature (22, 25, 33) reported an increase in the total and the acid-forming bacterial populations as a result of addition of sugar. More recent studies, however, showed that added sugar had little or no effect (55). Normal variation in bacterial population between duplicate barrels is probably responsible for this difference in results. For example, it has been reported

that a three-fold increase in acid-forming bacteria was present when sugar was added at the start of fermentation (55). However, no significance should be attached to such small differences in population.

When sugar was applied at the time of salting, no significant increase in yeast numbers was observed. Low populations were detected after 3 to 5 days of fermentation. During the same period acid formers were rapidly increasing. Apparently, yeasts which were introduced into the barrels by the cucumbers were being eliminated. As fermentation progressed, the change in the environmental conditions, such as salt concentration, and pH, were probably important factors. The most resistant yeasts survived, then started to multiply rapidly, and consequently the yeast population increased after about 7 days (Fig. 3, part B). When sugar was added during the active fermentation period on the 7th day, a marked increase in yeast population was observed. Evidently, yeast found a rich source of carbohydrate which they immediately utilized. When the reducing sugars of the brine were exhausted, a decline in yeast numbers occurred. Similar results were shown by Veldhuis *et al.* (55) under commercial conditions. They reported that when sugar was added on the 10th day, exceedingly high populations of yeast were detected on the 11th day, but the populations dropped rapidly to a very low figure on the 13th day. Application of sugar in three small portions at the time of salting, on the 7th day, and on the 14th day, resulted

in a significant increase in yeast population following the second addition of sugar. However, a doubtful increase was observed after the third portion of sugar was added. This was probably due to the presence of smaller amounts of reducing sugars in the brine. Consequently less yeast activity was found.

Experiments under optimum conditions show that utilization of reducing sugars was not enhanced by increasing the sugar concentration in the brine. As a matter of fact, L. plantarum was not able to utilize all the naturally occurring sugars in the cucumber juice (Table 8). Furthermore, when sugar was added L. plantarum counts showed no significant increase in numbers over the control. This coupled with the fact that lactic acid production did not increase indicates that, under the conditions of these experiments, the carbohydrate level in the brine was not a limiting factor for the growth of these organisms. Apparently, L. plantarum can only utilize a certain amount of sugar and produce a certain amount of acid irrespective of the amount of sugar present.

When studies of the cystine and tryptophane additions were carried out, results in general showed no significant effect of the treatments on the total titratable acidity and microbiologic activity.

In both the sugar and in the cystine-tryptophane treatments, all the vitamins and amino acids (except tryptophane),

which were examined, increased after salting, reached a maximum, then maintained high levels to the end of the examination period. Sufficient amounts were probably present at all times to satisfy the needs of acid-forming bacteria. These results agree with those of several other workers (11, 45). Costilow and Fabian (9) indicated that cystine might possibly be reduced to a critical level, if there is much coliform activity. Since Aerobacter fermentation in this study was insignificant, no such effect on cystine levels was observed. On the other hand, a marked reduction in tryptophane was consistently observed when yeast activity was greatest. It seems more likely, then, that tryptophane was utilized mainly by yeasts and not by the acid-forming bacteria.

When corn steep liquor was added, considerably larger amounts of acid were formed during the 3rd week of fermentation as compared to that of the control. Thereafter, a similar increase in acidity was observed. In addition, higher population of acid-forming bacteria was present, whereas, yeast numbers were significantly lower. During the same period, tryptophane did not decrease sharply. It maintained considerably high levels to the end of the examination period. Thus, it seems at first that the tryptophane concentrations might have an important role in acid production by the acid-forming organisms. However, addition of corn steep liquor in combination with sucrose stimulated acid formation, whereas,

the tryptophane levels were markedly lowered. Therefore, under the conditions of these experiments, tryptophane was probably not a limiting factor for the growth of L. plantarum. Apparently, greater yeast activity than in the case where corn steep liquor was used alone, was responsible for this reduction. Similar observations have been shown by Costilow and Fabian (9). In their experiments, tryptophane was reduced when great yeast activity occurred.

Results have also demonstrated that under semicommercial conditions, the utilization of the naturally occurring sugars in the cucumbers, was slightly more rapid when corn steep liquor was applied. Furthermore, under laboratory conditions, greater amounts of reducing sugars were utilized. Thus corn steep liquor might contain a substance or substances, other than those examined, which enhance greater and more rapid utilization of the natural sugars by the acid-forming bacteria. Another possibility might be that corn steep liquor provides a factor when its concentration declines to a critical level during the fermentation. Results, as previously mentioned, have shown that the effect of corn steep was first noted approximately during the 4th week of fermentation. Several workers have shown that corn steep liquor is a good source of soluble protein derivatives and minerals as well as growth promoting factors (2, 29, 48). In addition, Foster and associates (29) attributed the stimulatory effects of corn steep

liquor in the production of penicillin, due to the presence of arginine, histidine, glutamic acid and phenyl acetic acid derivatives. Since arginine, alanine, phenyl alanine and tyrosine have been shown sometimes to be stimulatory or essential for L. plantarum, further investigations along these lines would be of great interest (38).

It should be noted that the effect of corn steep liquor under laboratory conditions was more pronounced in the third flask experiment as compared to the fourth flask experiment. Thus, greater amounts of acid formation were detected (Tables 6 and 7). On the other hand it has been demonstrated before that an opposite relationship was observed for all other treatments. It seems, then, that the cucumbers used for the fourth experiment had less nutrients than those used for the third experiment. Apparently, corn steep liquor is more effective in promoting greater acid formation in a poor medium. Similar observations on the synthesis and utilization of some vitamins and amino acids in rich and poor media have been indicated (46).

SUMMARY

Studies on the effect of several treatments on lactic acid formation, the microbiologic activity and the availability of vitamins and amino acids in cucumber fermentations, were carried out in 1953 and 1954. Eighteen barrels were prepared. Seven barrels represented the sugar treatments, three barrels were used to study the effect of the cystine and tryptophane treatments, five barrels were used to investigate the effect of the corn steep liquor treatments, and three barrels were controls. Except for the corn steep liquor treatments, acid formation was not stimulated by the different additions of sugar, or cystine and tryptophane. When corn steep liquor was applied, however, acidity increased by at least 0.10 per cent over that of the control approximately on the 18th day and thereafter.

To investigate further the effects of these treatments, laboratory experiments were carried out under very favorable conditions for the growth of L. plantarum. Thirty-two flasks were prepared. Ten flasks were used for the sugar treatments, eight flasks for the cystine and tryptophane treatments, eight flasks for the corn steep liquor treatments, and six flasks were used as control. Only the corn steep liquor treatments were observed to stimulate lactic acid formation.

In some instances, the increase in acidity was as high as 0.4 per cent over that of the control.

Determination of the reducing sugars in different brines under semicommercial conditions indicated that the naturally occurring sugars in the cucumbers were rapidly utilized when corn steep liquor was applied. Under laboratory conditions, the effect of corn steep liquor was even more pronounced. Considerably greater amounts of reducing sugars were utilized by L. plantarum than in the control.

No significant effect of sugar, or cystine and tryptophane treatments on the total and acid-forming bacterial populations, was observed. In general, the numbers rapidly increased after salting, then reached a maximum from 3 to 6 days. A steady decline occurred thereafter. The corn steep liquor treatments showed similar maximum levels. However, the numbers declined at a slower rate than in the control with considerably higher numbers observed after 29 days and thereafter.

From the standpoint of yeasts, the added sugar or cystine and tryptophane resulted in no significant effect on yeast population when applied at the time of salting. In general, yeast declined after salting, started rapid growth about the 6th day, then reached a peak approximately between 10 and 15 days with little changes thereafter. However, when sugar was applied on the 7th day, the numbers rapidly increased to very high levels, but then rapidly dropped to a level comparable

to that of the control. The changes in the reducing sugars available in the brine indicate that yeast immediately utilized the added sugar, but decreased in numbers when the reducing sugars were depleted in the brine. All barrels where corn steep liquor was applied showed lower numbers of yeasts on the 18th day than the control. Similar observations were made during the rest of the examination period.

Under semicommercial conditions, the vitamins and amino acids (except tryptophane) which are essential for the growth of L. plantarum, increased after salting, reached a peak and maintained high levels throughout the rest of the examination period. Various treatments showed no effect different from that of the control on the levels of these vitamins and amino acids. Apparently, these vitamins and amino acids were present in sufficient amounts to satisfy the needs of L. plantarum. Tryptophane was the only amino acid affected by fermentation. Its concentration markedly decreased when yeast activity was greatest indicating utilization of tryptophane mainly by yeasts. In addition, tryptophane levels decreased at the end of the examination period in the control fermentation as well as in the brines where sugar or the cystine and tryptophane treatments were applied. Experiments with corn steep liquor alone and corn steep liquor in combination with sugar, indicated that under the conditions of these experiments, tryptophane probably was not a limiting factor for the growth of L. plantarum.

One can only conclude that in this study, the carbohydrates, cystine, and tryptophane, were present at all times in sufficient amounts for the growth of L. plantarum.

The possibility that corn steep liquor might contain a substance or substances, other than those examined, and which stimulated the acid-forming bacteria when its concentration was depleted in the brine, is indicated.

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