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CHEMICAL AND BACTERIOLOGICAL CHANGES IN DILL-PICKLE BRINES DURING FERMENTATION [1]

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The manufacture and storage of genuine dill pickles under climatic conditions typical of the southern states is generally unsatisfactory, and few southern pickle packers attempt to manufacture genuine or fermented dills.

In other sections of the United States several investigators have studied different phases of dill-pickle manufacture. Lesley and Cruess (1928) studied the relationship between brine acidity and soft pickles. Joslyn (1929), in extensive experiments, investigated the influence of numerous commercial practices on the quality of the finished product. Fabian and Wickerham (1935) have reported some chemical and bacteriological changes which take place in dill manufacture.

During the past few years a study of the manufacture of genuine dills under climatic conditions of eastern North Carolina has been undertaken. This paper is a report of a chemical and bacteriological study in regard to the types of microörganisms predominating, the utilization of sugar, and the production of acid during the fermentation. The data presented were obtained during the 1938 curing season.

EXPERIMENTAL PROCEDURE

In outlining the program followed in 1938 one treatment was considered standard; other treatments deviated somewhat from the standard with respect to brine salinity, added acid, or addition of sugar.

The cucumbers used were uniform in size and carefully selected. They were of 600-count size,[2] which would ordinarily be considered too large for marketable dills, but were entirely suitable for making comparisons of different treatments. Duplicate barrels of each treatment were put down.

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[2] Number per 45-gallon cask.

Brine samples were taken at frequent intervals for chemical and bacteriological analyses. These samples were taken by inserting a short piece of stainless steel tubing through the bung of the barrel and withdrawing the brine through an attached piece of rubber tubing. Two 12-ounce bottles full were withdrawn before the bacteriological sample was taken.

Chemical samples were preserved with sodium 2, 4, 5 trichlorphenolate in a concentration of 1-10,000 as described by Veldhuis (1938). The titratable acidity of the brine samples was determined by diluting a 10-c.c. sample with 50 c.c. of water, boiling about 20 seconds to expel carbon dioxide, cooling, and titrating with one-tenth normal sodium hydroxide using phenolphthalein as the indicator. The pH of the brine was determined by means of a glass electrode. Reducing sugars were estimated by the Shaffer-Hartmann (1921) micro method, standardized to the conditions under which it was used. Chemical studies were made on all lots.

For bacteriological analysis, the brine was examined by the plating technic with respect to total number of bacteria, acid-forming bacteria, peptonizing bacteria, and yeasts. Bacteriological findings showed the acid-formers and yeasts to be the predominating organisms and they alone are considered in this study.

The media used for plating of the brine samples were nutritive caseinate agar (Difco) for the bacterial counts and tartaric acid agar for yeast counts. The routine platings of the brine samples on the nutritive caseinate agar were counted and classified according to physiological growth reactions of the predominating organisms. On this medium the acid-formers showed definite zones of precipitated casein about the colony, owing to their acid production. The addition of eight c.c. of .4-per cent brom-cresol-purple indicator to the agar during preparation was a further aid in identification of the acid-forming bacteria. It was found that with the routine use of this media for brine platings, during the active phase of the dill fermentation substantially all of the colonies present on the plates were acid-formers. The yeast counts were obtained by plating dilutions of the brine on tartaric acid agar, which was prepared by adding five c.c. of sterile five-per cent tartaric acid to each 100 c.c. of melted dextrose agar.[3] All plates were incubated at 35°C.(95°F.) for three days and then counted. Occasionally, when yeast colonies were not well developed the incubation period was extended to five days. The treatments followed bacteriologically are outlined (Table 1, Nos. 2, 3, 5, and 11).

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[3] Laboratory Manual *(Methods of Analysis of Milk and Its Products)*, International Association of Milk Dealers, 1933. p. 81.

TABLE 1

Salting Schedule Followed in Experimental Dill Pack

(Treatment for all lots was the same as for the standard[1] except as otherwise indicated in this table)

Lot No.	Treatment description	Quantities of materials added per barrel		
		Salt	Acid	Sugar
		lbs.		
1	Standard	19	.5 gal. 110-grain vinegar	None
	Acid series			
	Vinegar—at starting			
2	No acid added	19	None	None
3	Medium acidity	19	1.0 gal. 110-grain vinegar	None
4	High acidity	19	1.5 gal. 110-grain vinegar	None
	Lactic acid— (vinegar omitted)	19		
5	At starting	19	1700 c.c. 50%	None
6	10 days after starting	19	1700 c.c. 50%	None
	Salt series			
7	Low salt	15	.5 gal. 110-grain vinegar	None
8	High salt	23	.5 gal. 110-grain vinegar	None
	Sugar series			
9	Dextrose at starting	19	.5 gal. 110-grain vinegar	3.5 lbs.
10	Dextrose 7 days after starting	19	.5 gal. 110-grain vinegar	3.5 lbs.
11	Sucrose at starting	19	.5 gal. 110-grain vinegar	3.5 lbs.

[1] Standard treatment—19 lbs. NaCl, .5 gal. 110-grain vinegar; barrel filled with water, stored inside shelter, rolled at frequent intervals early. All barrels were of 42-gallon capacity.

DATA AND DISCUSSION

Typical curves of chemical changes which occurred during fermentation in a lot receiving the standard treatment are shown (Fig. 1). Progressive changes in acidity and pH and sugar concentrations of the brine are indicated. From the acidity curve it is evident that the changes in this constituent were small during the first three days. The acid content of .4 gram per 100 c.c. at the end of the first day was due to the vinegar added. During the second and third days the brine acidity decreased somewhat as a result of brine dilution and vinegar absorption by the cucumbers.

A rapid increase in acid content began on the fourth and continued until about the 18th day, followed by a slight decrease, after which there were but small fluctuations in brine acidity.

The sugar curve (Fig. 1) indicates a rapid increase in brine-sugar concentration during the first three days and a correspondingly rapid reduction in this sugar concentration after an active fermentation had started, as indicated by the development of brine acidity.

The pH curve shows the effect of the added acid and acid produced by fermentation. The variations found with this treatment are not very large. The added vinegar lowered the pH to about 3.6 on the first day. During the second and third day the pH rose slightly as the vinegar in the brine was diluted. When the formation of acid by fermentation began, the pH decreased again and continued to decrease slightly as long as acid was formed. A constant value was approached on about the 18th day.

A more or less average dill fermentation is shown (Fig. 2) with respect to predominating microörganisms and their effect upon the sugar present and the production of brine acidity. The upper part shows the differential plate counts of the brine in regard to the acid-forming bacteria and yeasts. Owing to the great difference in the number of the two types, the counts are plotted logarithmically so that both may be shown on the same graph. The acid-forming bacteria show a growth curve of moderate activity, reaching a peak of 38 millions per c.c. on the third day and then subsiding. The yeast fermentation did not begin actively until the acid-forming bacteria had changed the acidity of the brine to more optimal conditions. The yeast population, after starting on the fourth day, reached a peak on the 10th day and then dropped sharply although remaining in the brine for approximately 30 days.

The lower part of Fig. 2 shows the reducing sugar content and titratable acidity of the brine during the fermentation. The acid-forming bacteria brought about the first part of the downward trend of the sugar curve, then the advent of the yeast fermentation aided in bringing about the further decline to approximately .1 per cent on the 10th day. The acidity curve, starting on the first day, shows a gradual rise to approximately .75 per cent on the 21st day, this period comparing favorably with that of the active growth phase of the acid-forming bacteria.

The influence of the addition of lactic acid and different quantities of 110-grain vinegar at the outset of the experiment on subsequent changes in acidity, pH, and sugar concentration of the brine during the fermentation is evident (Figs. 3 to 5). The details of these treatments are shown (Table 1, Treatments 1 to 5).

The quantity of lactic acid added for treatment No. 5 was equivalent to approximately one and three-fourths gallons of 110-grain vinegar. Since the dillweed used in these treatments had been packed in vinegar, a small amount of acid was introduced into all barrels including those designated as "no acid added." The amount of vinegar thus added was equal to about one pint of 110-grain vinegar.

For the vinegar-treatment series the curves (Fig. 3) show that there was a decrease in the acid content of the brines during the first few days as the vinegar became diluted. This condition was followed by a rapid rise in the acidity as acid was produced by the fermentations. The addition of successively larger amounts of vinegar at the outset had the effect of delaying the start of the acid fermentation for longer periods of time, but did not otherwise materially affect the formation of acid by fermentation. A half gallon of vinegar diluted to 42 gallons results in a solution having an acid content equivalent to about .2 gram of lactic acid per 100 c.c. The maximum acid content attained in lots with one-half gallon of added vinegar was approximately .2 gram per 100 c.c. more than that attained in lots receiving no vinegar; thus the increase was about equal to the added vinegar. In the cases where larger amounts of vinegar were added, the increase in the maximum acidities attained was roughly equal to the added vinegar.

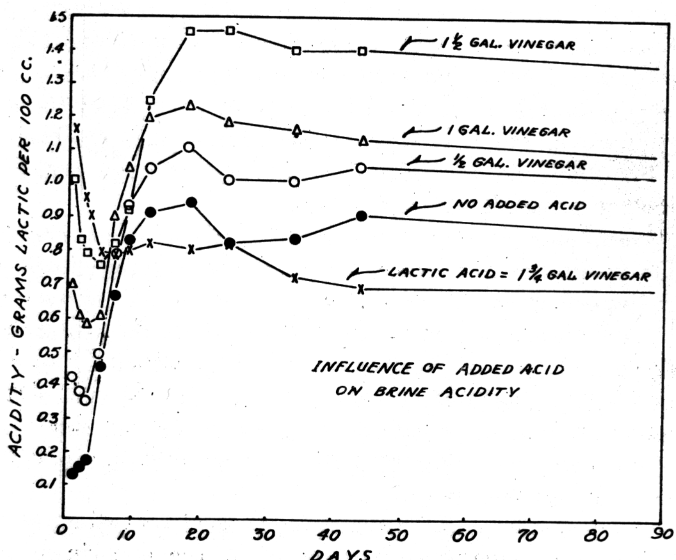

Fig. 3. Influence of addition of vinegar and lactic acid at the start of dill fermentation on acid formation.

The addition of lactic acid had an entirely different effect. Little or no acid was produced by fermentation in this case. The added lactic acid was sufficient to make a final acid content of .7 gram per 100 c.c. without any acid from fermentation and this was approximately the figure reached on the 14th day. In other words, acidity changes in this lot were practically restricted to the dilution of the acid added at the outset. Thus, the addition of lactic acid inhibited the normal production of acid by fermentation.

The curves for brine-sugar concentrations for the same treatments given in the acid curves (Fig. 4) are all similar in shape and show a rapid rise in the sugar content of the brines during the first three to five days followed by a rapid decrease until about the 18th day, at which time very little sugar remained. The main difference between the curves is the height of the curve for any given day. It is seen that the beginning of the utilization of sugar by fermentation was delayed by the addition of vinegar. The more vinegar added, the longer the delay. Regardless of the delay, once the fermentation started, there was little difference in the rate of disappearance of sugar from the brines of different lots. The difference in the maximum sugar content of the brines does not indicate that there was more sugar in the cases where vinegar was added, but rather that the utilization of sugar did not begin as soon. It is interesting to

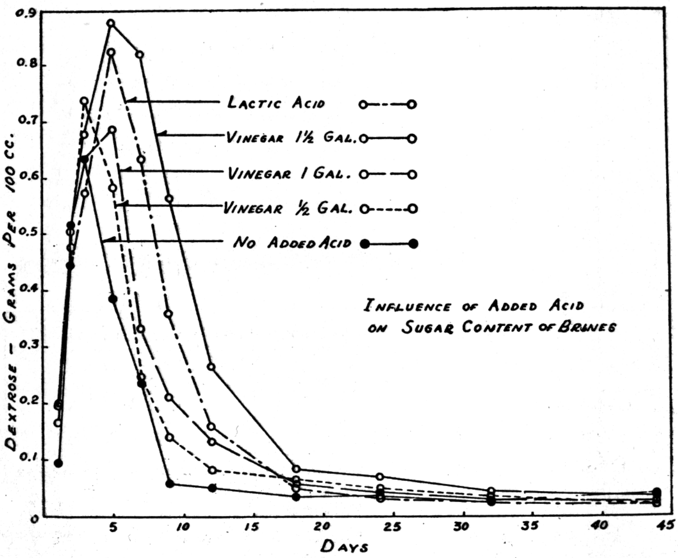

FIG. 4. Influence of the addition of vinegar and lactic acid at start of dill fermentation on disappearance of sugar.

note that the sugar curve for the lot with added lactic acid was similar to the other curves. The sugar was decomposed despite the fact that little or no acid was formed, as was shown by the previous diagram. The sugar was transformed into products other than acids.

The curves for pH for the same lots presented in the last two diagrams (Fig. 5) indicate that there is almost no difference in the values after the first 12 to 15 days; that is, the final pH was independent of the amount of acid added or the acidity developed in the ranges employed in these experiments. The greatest differences appeared during the first 12 days, at which time the main part of the acid formation was taking place. The additions of vinegar reduced the pH from an initial value of 6.9 to from 3.4 to 4.1 on the first day followed by a slight rise in pH as the vinegar became diluted. After two to five days a decrease in pH occurred owing to the production of acid by fermentation. Successively larger amounts of vinegar caused a longer delay before acid formation by fermentation occurred. The curve for the lot with added lactic acid is obviously unlike the curves for the vinegar series. Apparently the only effect of adding the lactic acid was the rise in pH as the acid became diluted.

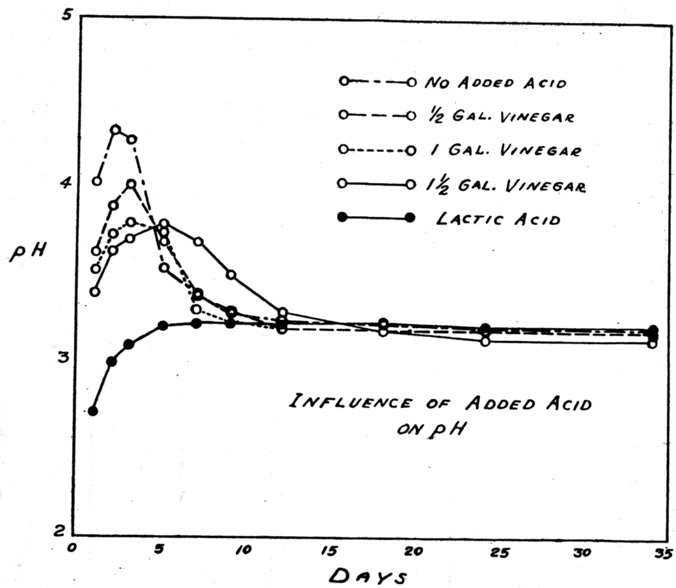

FIG. 5. Influence of addition of vinegar and lactic acid at the start of dill fermentation on brine pH.

The influence of the above-mentioned treatments involving added vinegar and lactic acid (Table 1, Treatments 2, 3, and 5) on microbiological activity during the fermentation is shown (Fig. 6). All treatments were in duplicate and data shown are averages. The results are shown with respect to the predominating microörganisms (upper part) and chemical analyses for reducing sugars and titratable acidity (lower part). It will be noted that the acid-forming bacteria counts reached 100 million per c.c. on the fifth day in the case of the fermentation without added acid and then declined to below the 100,000-per-c.c. range in 30 days. The addition of one gallon of 110-grain vinegar exerted a retarding effect on the lactic fermentation and in general resulted in somewhat lower bacterial counts. The inhibiting effect of lactic acid (equal to one and three-fourths gallons of vinegar) is evident, the counts being wholly within the 10,000-per-c.c. range. It will be noted also that the fermentation receiving lactic acid showed vigorous activity with respect to yeasts, considerably higher populations than that of the fermenta-

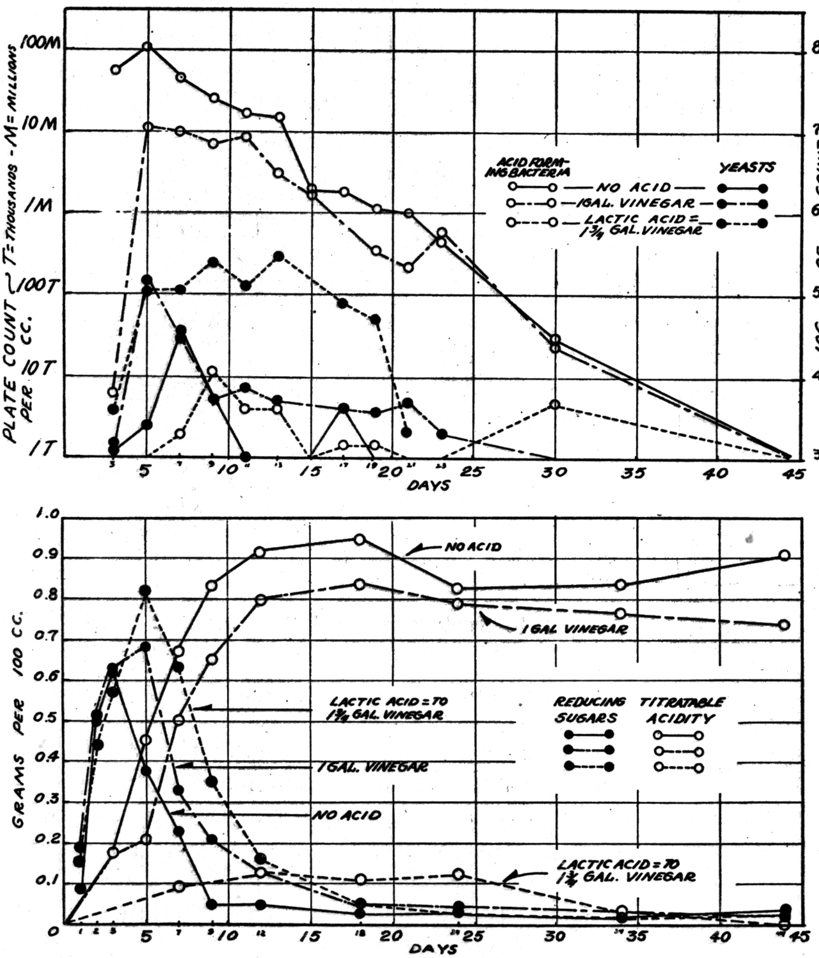

FIG. 6. Growth of predominating microörganisms as influenced by the addition of vinegar and lactic acid at the start of dill fermentation. Upper part, growth curves of acid-forming bacteria and yeasts; lower part, sugar utilization and acid formed by fermentation.

tion receiving one gallon of vinegar. The fermentation receiving no added acid had lower population of yeasts and they appeared for a shorter time than either of the fermentations receiving lactic acid or the one receiving vinegar.

The rate of sugar utilization, as indicated by the decline of the sugar curves, was related to the numbers of the microörganism present in the respective brines at any given time. A sharp decline in sugar content was first observed in the fermentation with no added acid, followed by the fermentation receiving the vinegar.

The lactic acid treatment was the last to show marked sugar utilization. Since there were few acid-forming bacteria in the lactic acid treatment at any time and since the yeast fermentation did not begin vigorous activity until the fifth day, it would be expected that the sugar utilization would be retarded. In the no-acid treatment the fermentation developed approximately .95 per cent acid, while the fermentation receiving one gallon of 110-grain vinegar

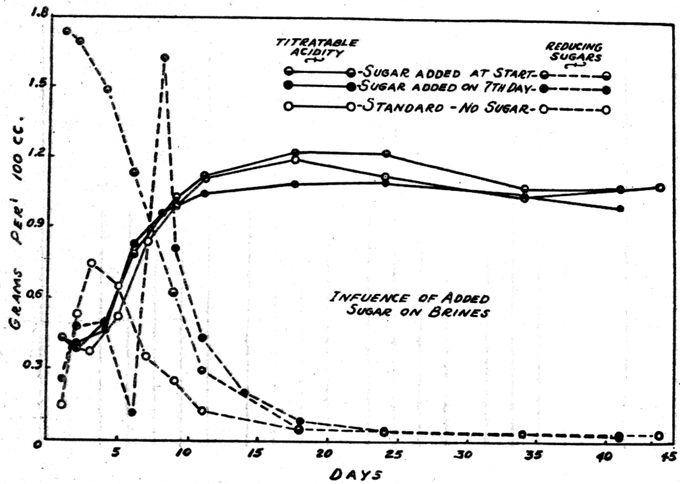

FIG. 7. The effect of added sugar on the acid formation in dill brine.

produced approximately .82 per cent. The lactic acid treatment, which inhibited the growth of acid-forming bacteria, produced approximately .1 per cent acid.

The relationships are shown (Fig. 7) of sugar concentrations and acidity developed in lots receiving standard treatment compared with lots to which three and one-half pounds of sugar were added at the outset and other lots to which the same amount of sugar was added on the seventh day. In spite of the addition of sugar, there was little or no difference in the amounts of acid produced. Lots to which the sugar was added at the outset produced slightly more acid than the standard treatment and the lots which received the sugar on the seventh day produced less acid, but the variations are not much greater than those that would normally be expected within the same treatment. It is particularly interesting to note that the great differences in the sugar content of the brines from the fifth to the 10th day had little or no effect on the rates of acid formation.

The effect of the addition of three and one-half pounds of sugar at the start of the fermentation on predominating microörganisms is shown (Fig. 8) and the results are compared with another fermentation, started at the same time and under like conditions but having no sugar added. Both fermentations received one-half gallon of 110-grain vinegar as standard treatment. Examination of the acid-forming bacteria growth curves (upper part Fig. 8) shows a sharp

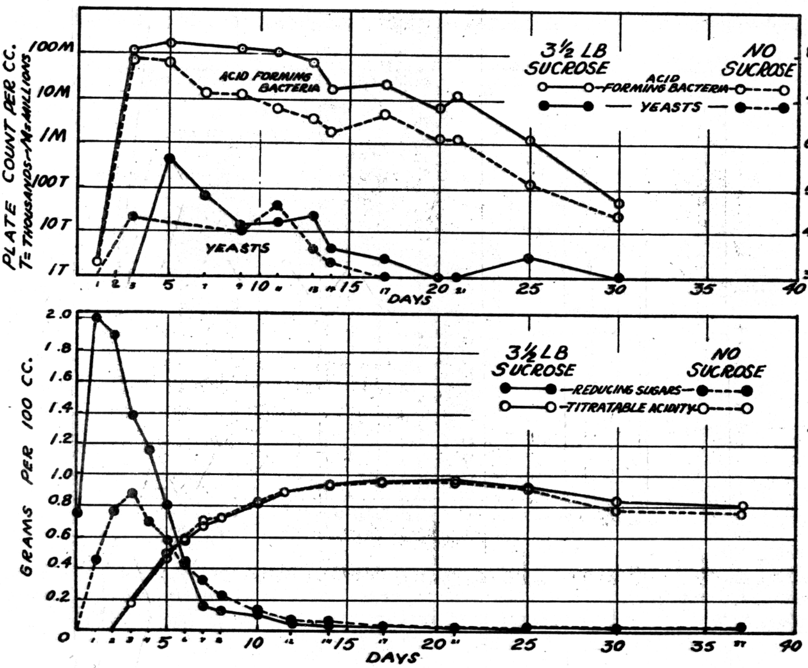

FIG. 8. Effect of the addition of sugar at the start of dill fermentation on growth of predominating microörganisms. Upper part, growth curves of acid-forming bacteria and yeasts; lower part, sugar utilization and acid formation.

rise to 110 millions per c.c. for the fermentation receiving sugar and 70 millions per c.c. for the one receiving no sugar at the five-day plating interval. At this point the growth curves separate and during the major part of the fermentation period the counts from the brine receiving sugar were approximately ten times as great as those from the brine receiving no sugar.

The yeast counts show that in the case where sugar was added the yeast population, in general, was considerably higher and of longer duration than in the case where no sugar was added.

The sugar analyses (lower part Fig. 8) show that in both fermentations the utilization of sugar reached a similar figure after 10 days. It will be noted that there was little or no difference in the amount of total acid produced, indicating that the addition of sugar, while resulting in an approximate ten-fold increase in population of acid-forming bacteria, did not result in corresponding increase in acidity.

A comparison in diagrammatic form is shown (Fig. 9) of all the lots cured according to the schedule outlined in Table 1. In all treatments the brine acidity reached its highest value during the second week of fermentation, and the values for the 18th day generally represented the maximum acidity attained with each treatment.

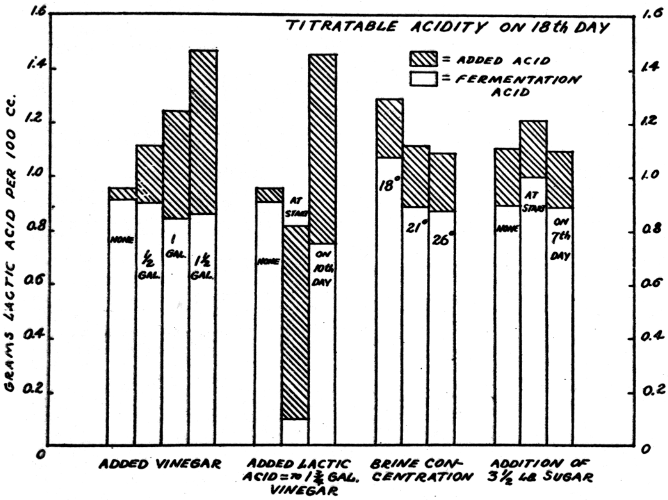

Fig. 9. Summarization of influence of different treatments on amount of acid developed during dill fermentation. Total height of column represents total brine acidity, height of unshaded portion represents acid formed by fermentation.

For the vinegar series this diagram indicates that the addition of vinegar did not materially affect the quantity of acid produced by fermentation.

The values for the lactic acid series demonstrate that the addition of this acid at the outset of the experiment inhibited the production of acid by fermentation processes. However, the addition of lactic acid on the 10th day resulted in a high brine acidity because a normal fermentation had occurred during the period prior to the addition of lactic acid.

The use of brines of different salt concentration resulted in variations in quantity of acid formed. The greater acid production occurred in the brines of lower salt concentration, and lesser acid production in the brines of higher salt concentration, as shown previously by Jones (1940).

The addition of three and one-half pounds of sugar at the outset of the experiment resulted in an increase in the titratable acidity equivalent to about .1 per cent lactic acid; the addition of the sugar on the seventh day had practically no effect. This indicates that the addition of sugar to the brines did not appreciably increase brine acidity.

The presence of hollow cucumbers or "bloaters" is frequently encountered in the manufacture of dills. In this study some striking differences in the number of bloaters formed during the curing process were observed. Since the cucumbers were large (600 count), the percentage of those found to be hollow was somewhat higher than

TABLE 2

Influence of Some Factors on the Formation of Hollow Pickles or "Bloaters" in Dill-Pickle Manufacture

Treatment	"Bloaters"
	pct.
Standard	20
High salometer (26°)	45
Lactic acid (= 1¾ gal. vinegar) added at start	67
Sugar added at start, 3½ lbs.	78
Sugar added on seventh day, 3½ lbs.	70

might have been expected if cucumbers of the usual dill size had been used. For most treatments the number of bloaters ranged from 10 to 25 per cent. The treatments which favored the development of bloaters in considerably greater numbers than the standard are shown (Table 2).

SUMMARY AND CONCLUSIONS

Various methods of manufacture of dill pickles under southern conditions have been tested. The effects of brine salinity and the addition of vinegar, lactic acid, and sugar upon fermentation were determined by chemical and bacteriological analyses of the brine during the curing process. Observations of the influence of some of these factors on "bloater" formation have been made. The results of these studies may be summarized according to the various treatments as follows:

Vinegar added at the start of the fermentation delayed acid formation but did not appreciably influence the quantity of acid produced or the rate of disappearance of sugar from the brine.

The addition of lactic acid inhibited the normal production of acid but did not markedly affect the rate of sugar utilization. Lots receiving lactic acid at the outset of the experiment gave high percentages of bloaters while those lots receiving the acid after 10 days gave moderate percentages of bloaters.

The addition of sugar did not significantly increase the production of acid by fermentation, although there was a rapid utilization of sugar. However, the addition of sugar resulted in a marked increase in the percentage of bloaters formed.

Increases in brine salinity resulted in slightly decreased total acid formation but did not otherwise markedly affect the production of acid or utilization of sugar. However, brine concentrations much above 21° salometer are not usually satisfactory since the finished product is too salty for edible purposes.

Under the conditions which prevailed during the course of these experiments the predominating microörganisms in dill-pickle fermentations were found to be acid-forming bacteria and yeasts. Peptonizing bacteria were also recognized in the flora of the dill fermentation. These latter appear in relatively small numbers, however, and may be wholly absent, particularly in cases where acid is added at the start of the fermentation.

The acid-forming bacteria were present in the greatest numbers, millions per cubic centimeter, within two to four days and remained in high counts for approximately two weeks. Following this period their numbers decreased although they were present in the brine for about 30 days.

The yeast fermentation was found to be common to all dill fermentations studied. This is of particular significance inasmuch as the definite rôle of yeasts in dill fermentations has been, heretofore, generally unrecognized. The yeast fermentation occurred after the acid fermentation had reached a definite activity. It was more irregular in its course, in some instances, than the acid fermentation. As indicated by plate counts the yeasts are present in decidedly lower numbers per cubic centimeter than acid-forming bacteria.

It was found that the addition of sugar to a dill fermentation resulted in a marked increase in population of acid-forming bacteria, with no appreciable increase in final acidity. Also, the yeast population was somewhat higher in the fermentation to which sugar was added.

The addition of one gallon of 110-grain vinegar to a dill fermentation showed an inhibiting effect upon the population of acid-forming bacteria but did not significantly influence the quantity of acid produced by fermentation as compared with a treatment without added vinegar. When vinegar was added, the yeast fermentation started sooner and showed moderately higher counts than a comparable fermentation without vinegar.

A marked inhibition of the acid-forming bacteria was evidenced by the addition of lactic acid to a dill fermentation. The counts of these organisms were found to be decidedly lower than in the case where added vinegar was employed. The use of lactic acid resulted in a most active yeast fermentation of prolonged duration, accompanied by high counts as compared with other yeast fermentations.

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