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INCIDENCE OF YEASTS IN CUCUMBER FERMENTATIONS[1,2]

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While numerous investigators have carried out experimental work over a widespread area and under varied experimental conditions in regard to the microbial flora occurring during the fermentation of cucumbers, little or no attention has been paid to that phase of the fermentation proper which is brought about by true yeasts. In fact, the rôle of yeasts in the fermentation of cucumbers has been more or less unrecognized by workers in this field. This is particularly interesting in view of the fact that in several cases yeasts are mentioned in the literature—Kossowicz (1909), Hasbrouck (1910), LeFevre (1922), and Vahlteich, Haurand, and Perry (1935)—and in some instances in connection with evolution or bubbling of gas during fermentation—Riley (1914), Brown (1916), and LeFevre (1921)—yet no systematic study as to their populations in the brine is reported to show definite association with cucumber fermentation.

It has been the aim of the present study to deal specifically with yeast populations in brines of different initial salt concentrations; the latter being a part of a general program being carried out on the fermentation of cucumbers for salt stock production. The work covers a four-year period (1936 to 1939) and was carried out under what could be called commercial salting conditions. The major portion of the fermentations was in vats of approximately 85-bushel capacity; all vats were outside, thus reducing to a minimum pseudo-yeast (Mycoderma) growth.

EXPERIMENTAL PROCEDURE

In general the salting procedure was as follows: Vats were filled with cucumbers, headed, and salt brine was added so as to come a few inches above the heads. The actual salting schedule for the different initial brine concentrations, and the rate of increase in brine concentration for the treatments are shown (Table 1). Initial brine

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[2] In coöperation with the Department of Horticulture, North Carolina Agricultural Experiment Station.

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concentrations of 20, 30, and 40° [4] were employed as regular treatment in vats of 85-bushel capacity. One treatment other than regular was given two vats of 180-bushel capacity salted at 30° where the salt and water were added, during the filling of the vats with cucumbers, in amounts sufficient to make an initial 30° brine. In all a total of 24 vats was followed with respect to yeast populations. The four 40° treatments followed during the 1936 season were used as a basis for the later studies and were on large vats (720 bu.).

Brine samples for analysis were taken as follows: For the preliminary work (1936) the samples were taken by use of a stoppered

TABLE 1

Salting Schedule for 1937, 1938, and 1939 Seasons

Initial brine concentration	Rate of increase of brine concentration [1]	Vats	
		Number salted	Capacity
			bu.
Regular Treatment			
20°	Up 10° per week to 60°	6	85
30°	Up 7.5° per week to 60°	4	85
40°	Up 5° per week to 60°	8	85
40° [2]	Up 2° per week to 50° then 5° per week to 60°	4	720
Not Regular Treatment			
30° [3]	Up 7° per week to 70°	2	180

[1] After the first week. [2] Treatment used for preliminary work during the 1936 season. [3] Salt and water added during filling of vats with cucumbers.

bottle fastened to a long, narrow piece of pine siding, the latter being run down the sample slot to a midway depth in the brine and the stopper removed by an attached cord. The above sampling method was improved for the following seasons and samples were taken by inserting a piece of stainless steel tubing through an opening in the head of the vat into the brine toward the center of the vat and withdrawing the brine sample through an attached piece of rubber tubing. Two 12-ounce bottles of brine were withdrawn before taking a sample for analysis. Samplings in all cases were started at the time the vats were put down and headed and were continued at intervals during the course of fermentation. Fermentations occurred for the most part during the month of July with the brine temperature approximately 26.7°C.(80°F.).

Yeast populations in the brine were determined by plating dilutions of the brine on tartaric acid agar.[5] In brief, this medium

[4] Per cent saturation with respect to sodium chloride.

[5] Laboratory Manual (Methods of Analysis of Milk and Its Products). Int. Assoc. Milk Dealers, p. 81 (1933).

consisted of ordinary dextrose agar to which five c.c. of sterile five-per cent tartaric acid were added to 100-c.c. amounts of the agar prior to pouring the plates, the addition of the tartaric acid bringing the pH of the medium to approximately 3.7 thus withholding all of the usual brine organisms but yeasts.[6] The yeast plates were usually incubated for three days at 35°C. (95°F.); in cases where growth was inadequate, the incubation period was extended to five days.

PRELIMINARY EXPERIMENTS

The four 40° vats salted during the 1936 season served as preliminary experiments with regard to analyses of the brine for the presence of yeasts. Two of this group (Vats 124 and 126) were put down on the same date (6-30-36), the remaining two (Vats 125 and 122) followed at intervals of six and seven days, respectively.

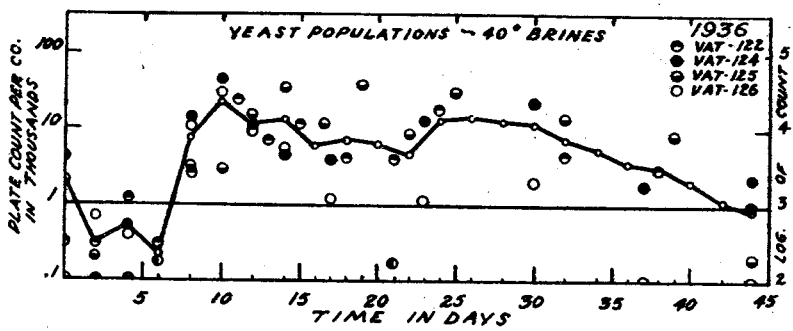

FIG. 1. Yeast-fermentation trend in 40° brines during 1936.

The yeast fermentation trend for all vats is shown (Fig. 1) as the arithmetic mean of the plate counts for all vats and the values are plotted logarithmically at regular intervals during the period of analyses. On the initial day (the time the vats were salted) the analyses showed yeasts were present in numbers slightly over 1,000 per c.c.; they remained in the brine in numbers less than 1,000 for about a week when active yeast fermentation began. It will be noted that the active phase of the yeast fermentation, after having started in about one week, followed a somewhat irregular course with respect to numbers for about 30 days, after which time the counts declined more or less gradually to the 1,000 per c.c. range. In general, the mean populations were in the neighborhood of 10,000 per c.c. during the period from the eighth to the 32nd day. Considerable variation

[6] If this medium is used for analyses of brines sheltered from direct sunlight or otherwise receiving no scum control, pseudo-yeasts (Mycoderma) will grow out and are apt to be confusing.

is noted between fermentations. In the case of Vats 124 and 126 very low counts on the 21st day accounted for lowering of the general fermentation trend during the period from about the 16th to 22nd day. However, the most important result to be noted from

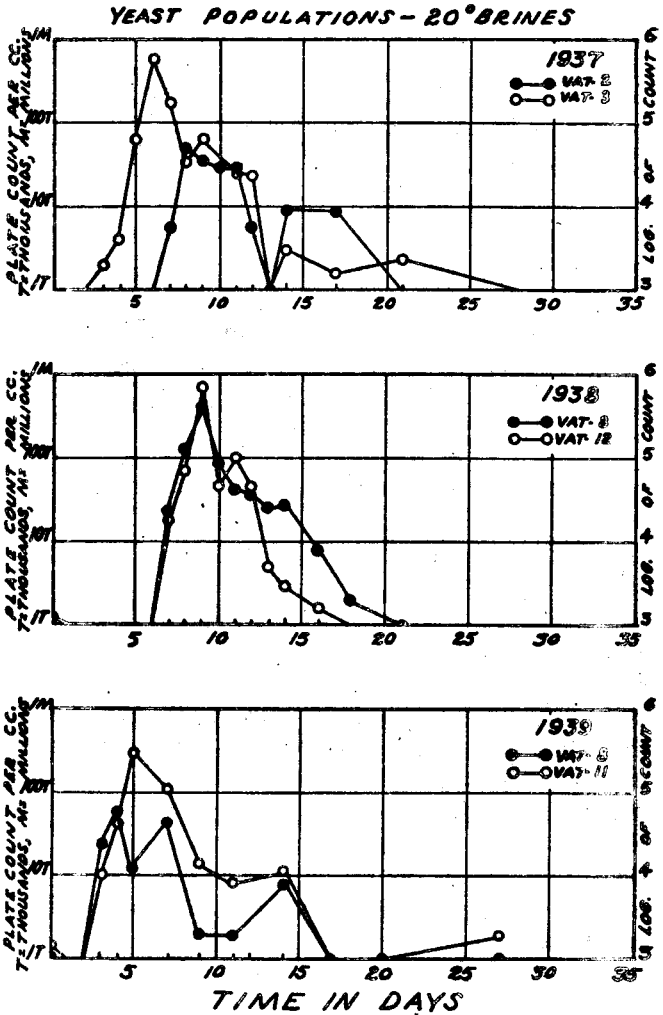

FIG. 2. Growth curves for yeasts in 20° brines for a three-year period (1937-1939).

these preliminary observations is the fact that yeast populations, showing a definite fermentation trend, were established as a part of the cucumber fermentation proper.

DISCUSSION OF RESULTS

Following the preliminary work of 1936, observations were made with respect to yeast populations in brines of 20, 30, and 40° treatment during the next three salting seasons. All fermentations were in vats of 85-bushel capacity and an improvement in brine sampling was effected. Also, an effort was made to put down vats at the same time, particularly vats receiving the same salting treatment. The results are shown in respect to brine treatment (20, 30, and 40°) with the number of vats followed during each year shown as a separate unit. Plate counts made at the sampling intervals are plotted logarithmically to facilitate yearly comparison where counts vary greatly, as well as individual variations within the same year. The lowest values plotted are counts of 1,000 per c.c.

20° BRINES

Yeast populations in the 20° brines for the three-year period are shown (Fig. 2); initial counts above 1,000 per c.c. are shown in three cases. Where no counts are shown the yeasts were usually present but in numbers less than 1,000 per c.c. In general, yeast fermentation started in about three to six days, reached a peak (50,000 to 600,000 per c.c.) within the first week and then declined. Counts decreased to the neighborhood of 1,000 per c.c. after a period of about three weeks. Variations are evident within the same year as well as between different years.

30° BRINES

Analyses of 30° brines with respect to yeast populations were carried out on the regular treatment for a two-year period (1938 and 1939). These are shown in the upper part and middle part of Fig. 3 and are discussed first. Both 30° fermentations during the 1938 season showed a somewhat irregular course, subjected to peaks of high and low counts. Active yeast fermentation began after about one week and reached the first peak on the 11th day (approximately 50,000 per c.c.) for both vats. However, similar peaks were again noted on the 13th and 16th days for Vats 13 and 9, respectively. Counts decreased to less than 1,000 per c.c. after 21 days for Vat 9 and after 28 days for Vat 13.

The yeast fermentations in the vats followed during the 1939 season (middle part, Fig. 3) were quite similar in character. Both showed counts in the 1,000 per c.c. range at the initial analysis. After beginning activity on about the fifth day, they showed a course of active fermentation that extended to the 17th day, at which time counts declined to 1,000 per c.c. in both cases. The yeast count reached a maximum in one case (Vat 1) of about 400,000 per c.c.

on the seventh day and in the other case (Vat 3) of about 175,000 per c.c. on the 11th day.

In general, comparison of the yeast fermentations for the two seasons would indicate a more regular course of fermentation with

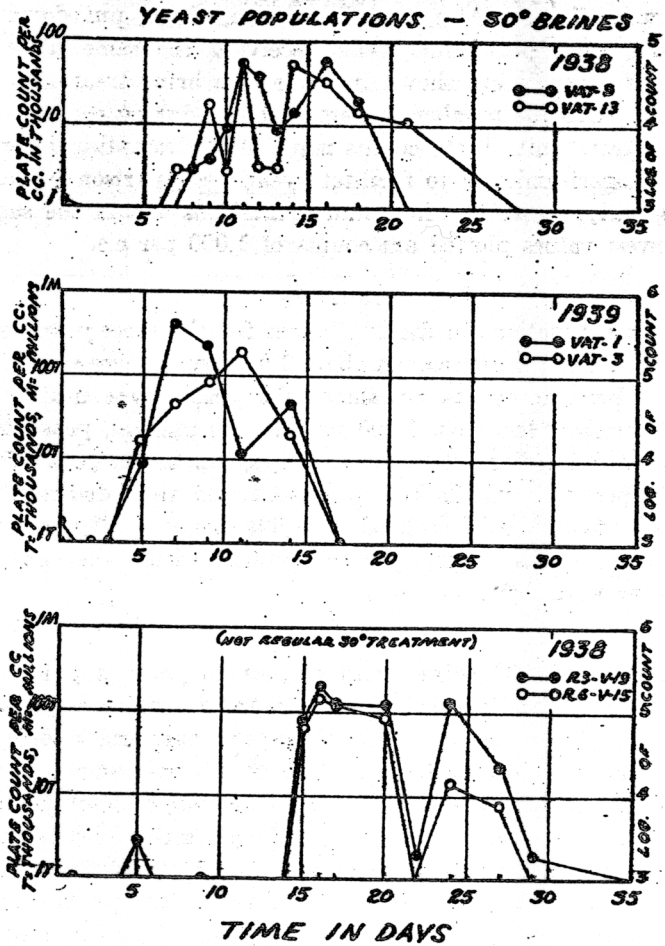

FIG. 3. Upper and middle parts, growth curves for yeasts in regular 30° brines for a two-year period (1938-1939); lower part, growth curves for yeasts in 30° brines not receiving regular treatment (1938).

somewhat higher maximum counts for the 1939 season. The duration of active yeast fermentation for both seasons is not particularly different.

The influence of a different 30° salting treatment (Table 1) on yeast populations, based on one season's observations, indicate a considerable difference when compared with regular 30° treatment pre-

viously described. The most striking variation is the length of time (about two weeks) required for the yeast fermentation to start in the 30° brines not receiving regular treatment (lower part, Fig. 3). It will be noted that the yeast populations in both cases (Vats R3-v19 and R6-v15) rose sharply to near the 100,000 per c.c. range on the 15th day and remained in or above that range until the 20th day, then declined sharply. After a second rise and fall, counts in about the 1,000 per c.c. range were reached on the 30th day.

40° BRINES

The growth curves of the yeast fermentation of 40° brines (Fig. 4) are similar in some respects to those of brine concentrations previously discussed. They are, however, singularly different in respect to the length of time which yeasts occur in 40° brines as compared with 20 and 30° brines. In the majority of the cases of the 40° brines, yeast counts of 1,000 per c.c. are noted after a period of 28 days. Generalized observations for all three years show that usually most of the yeast fermentations started after about one week, reached maximum counts (100,000 per c.c. range) after 13 to 17 days, then declined to the 1,000 per c.c. range after a fermentation period of about 30 days. The above observations are not without exception, however, as will be noted in the case of the 1939 observations (lower part, Fig. 4). Here one of the yeast fermentations started three to four days after the vat was put down. Also, the maximum counts in both fermentations were considerably higher than those of the two preceding years.

20 AND 40° FERMENTATION TRENDS

The fermentation trends for all 20 and 40° brines are shown (Fig. 5) with respect to yeast populations. The values plotted represent the geometric mean of the counts taken at two-day intervals from Figs. 2 and 4 throughout the yeast fermentations. Counts less than 1,000 per c.c. are shown below the double line and are connected by dotted lines. The mean values are plotted logarithmically, principally to permit showing yeast counts during the period prior to active yeast fermentation. No counts less than 100 per c.c. were included in the calculations.

In the case of the 20° brines, the initial presence of yeasts in relatively low numbers is shown. After a period of about three days of inactivity the typical yeast fermentation ensued and reached a peak on the ninth day, then declined so as to reach the 1,000 per c.c. range on the 18th day at which time active fermentation was considered to be complete. Yeasts are shown to be present in the brine in relatively low numbers, however, until the 26th day.

The fermentation trend for the 40° brines shows yeasts initially present in numbers less than 1,000 per c.c. and remaining in that range for approximately one week. After this period the counts rose so as to reach a peak on about the 14th day and then declined so that the yeast fermentation was more or less complete after 28 days.

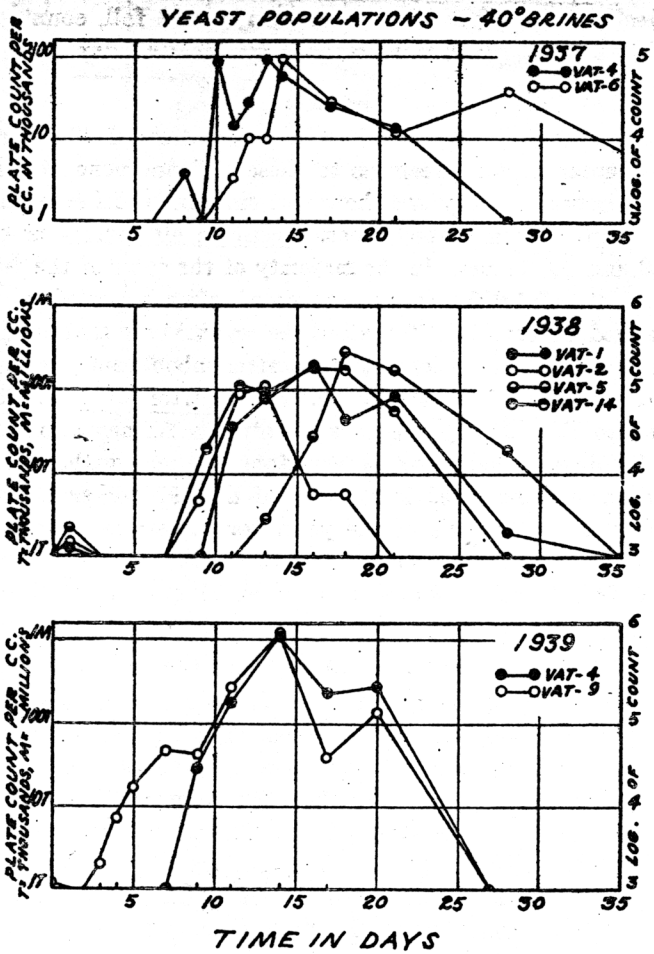

FIG. 4. Growth curves for yeasts in 40° brines for a three-year period (1937-1939).

The observations indicate that the probable active period of yeast fermentation in 20° brines would be about 15 days, while in the 40° brines it would be about 20 days. Also, it is evident that in general, the 20° brines would begin active fermentation somewhat sooner (about five days) as compared with the 40° brines.

A comparison of the maximum numbers of yeasts observed in all 20 and 40° brines would tend to show that the latter brines had a relatively higher figure (about 100,000 per c.c. compared with 50,000). It will be noted from individual cases, however, that while the time of starting of active yeast fermentation and the length of fermentation show remarkable correlation when compared, the maximum counts of the yeast present do not necessarily show the same relationship. The counts per cubic centimeter may, in fact, show as much as a ten-fold difference in like brine concentrations during the same season's observations as well as for that of different seasons.

The seeming lack of direct correlation between brine concentration and maximum numbers of yeast present is emphasized further

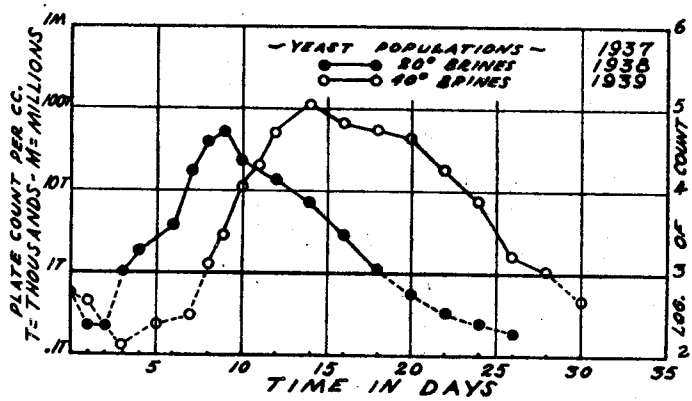

FIG. 5. Yeast-fermentation trends in all 20 and 40° brines over a three-year period (1937-1939).

in observations made on 60° brines. In two cases during the 1939 season yeast populations of 1,000,000 per c.c. were observed.

The discussion of fermentation trends has been limited to yeasts in 20 and 40° brines; 30° treatments have not been dealt with owing to lack of a comparable number of observations. However, it seems reasonable to assume that the general fermentation trend for 30° brines would probably fall between the 20 and 40° trends, being somewhat more similar to the former.

DISCUSSION

This study has been concerned with yeast populations in brines receiving different treatments with respect to salt concentration; however, it is evident that the latter governs to a greater degree the time that active yeast fermentation begins and the duration of that fermentation than it does the yeast populations once the fermentation is under way.

In the lower salt concentration brines, an earlier lactic acid fermentation in a relatively short time brings about brine conditions which are more favorable in respect to pH for yeast growth. In the higher salt concentrations, the acid fermentation being somewhat delayed, also delays the beginning of the yeast fermentation. Also, the duration of the active phase of yeast fermentation is affected to a great extent by the amount of food material available at the time the yeast fermentation starts. A vigorous, early acid fermentation in 20° brines would no doubt utilize the food material more rapidly than would the slower acid fermentations in the higher brines (40°).

The influence of the lactic acid fermentation in brines of different salt concentration upon the yeast fermentation will be presented at a later date.

SUMMARY

Under the conditions described for salting cucumbers, a part of the resulting fermentation is brought about by yeasts.

Active yeast fermentations were found in brine treatments of 20, 30, and 40 per cent saturation with respect to salt.

A definite correlation was found between brine concentration and the beginning and duration of the yeast fermentations (20 and 40° brines).

REFERENCES

BROWN, C. W., 1916. Notes on brine pickle fermentation. J. Bacteriol. 1, 104.

HASBROUCK, F. F., 1910. Salting and curing of cucumber pickles. Pure Products 6, 509-514.

KOSSOWICZ, A., 1909. Die Schungarung eingesauerter Gurken und die Anwendung von Reizuchten von Milchsaurebakterien bie der Gurkensauerung. Ztschr. f. Landwirt. Versuch. in Oesterreich 12, 757-770.

LEFEVRE, E., 1921. Bacteriology of pickle fermentation. The Canner 53, 39-40.

———, 1922. Bacteriology of sauerkraut and pickles. Chemical Age 30, 24.

RILEY, H. N., 1914. Preliminary notes on the curing of cucumber pickles. Science, n.s. 39, 954.

VAHLTEICH, H. W., HAURAND, C. H., AND PERRY, C. A., 1935. Modern science applies itself to cucumber salting. Food Indus. 7, 334-336.