

Bacteriological Changes In Cucumber Fermentation*

By JOHN L. ETHELLES, Agricultural Chemical Research Division, USDA, Raleigh, N. C., and
IVAN D. JONES, North Carolina Agricultural Experiment Station, Raleigh, N. C.

Research is now being conducted by the authors on brine pickling of vegetables. Any development in this field of food preservation must depend upon an understanding of the bacteriological changes involved. This article presents results of several years' work on the conditions prevailing during the fermentation of cucumbers

BRINE fermentation of cucumbers for salt stock purposes utilizes the selective action of the salt brine on the naturally occurring microorganisms to promote a fermentation resulting in the production of acid.

Using the general commercial salting methods, an acid fermentation commences shortly after cucumbers are brined and continues for from two to six weeks. The initial inoculum for the fermentation comes from the cucumbers and from adhering particles of soil. Salt-tolerant organisms utilize as their nutritive material the soluble constituents that diffuse into the brine as the result of the action of salt on cucumber tissue. They bring about the production of various compounds, chiefly lactic acid, but also acetic acid and alcohols, as well as the evolution of considerable quantities of gas. The formation of these end products was discussed in detail in the January issue of *FOOD INDUSTRIES*, page 62.

The curing process, requiring about three months, is one of complex bacteriological, chemical and physical changes. Any one of these changes constitutes an adequate basis for considerable fundamental investigation. Such studies would go a long way in placing the pickling industry in the group of scientifically controlled fermentations.

For the past 40 years, research in this field has dealt principally with the bac-

teriology of the lactic acid fermentation, with a very small proportion of the work being carried out under plant conditions. Little or no attention has been given to the probability that fermentations due to microorganisms other than lactic acid forming bacteria exist and contribute to the general fermentation. The true yeast fermentation which is associated with the general cucumber fermentation was not reported by workers in this field until recently^{1,2}. Similarly, combined bacteriological and chemical studies have demonstrated that still another phase should be included in the fermentation proper^{3,4}. This phase is brought about by a group of gas-producing, salt-tolerant microorganisms belonging to the genus *Aerobacter*. Such fermentation can be detected easily, because the evolved gas in composed of approximately equal parts of carbon dioxide and hydrogen.

Cooperative investigations were conducted at one of the large pickling plants in Eastern North Carolina from

about the middle of June until the last of August. Bacteriological observations reported were made chiefly on fermentations in vats of 85-bu. capacity, salted under outside conditions. The average brine temperature during the fermentation period was about 80 deg. F. Three salting schedules were followed, with initial salt concentrations of 20, 40 and 60 deg. sal. The 20-deg. brine was increased 10 deg. per week for five weeks and the 40-deg. brine, 5 deg. per week for four weeks. The 60-deg. brine was held constant. All brines thus were at 60 deg. after the first month or five weeks of curing. Salting treatments are designated throughout by the initial salt concentrations, expressed in "degrees salometer."

The bacteriological analyses were carried out with respect to populations of acid-forming bacteria, yeasts and hydrogen-producing bacteria belonging to the genus *Aerobacter*. The methods of examination of brine fermentations with respect to the first two types of organisms have been fully described in previous reports.^{5,6} Estimates of the numbers of the *Aerobacter* group were made by plating known dilutions of the brine on brilliant green agar, as well as by adding serial dilutions of the brine to lauryl-sulphate broth. Comprehensive preliminary studies indicated that the use of differential media was sufficient to obtain a reasonable estimate of the numbers of the *Aerobacter* group present in brine fermentations.

General Relationships in Fermentations Due to *Aerobacter*, Acid-forming Bacteria and Yeasts in 20-, 40- and 60-Deg. Brines*

Organisms	Brine conc. (dig. sal.)	General type of fermentation	Fermentation activity	Chief products formed	Approximate start of fermentation**	Approximate duration of fermentation
<i>Aerobacter</i>	20	Flash-gaseous	Slight, may be absent	Hydrogen and CO ₂	1st day	2-3 days
	40	Gaseous	May or may not be active	Hydrogen and CO ₂	1st-3rd day	1 week
	60	Gaseous	Usually active	Hydrogen and CO ₂	7th-8th day	1 week
Acid-forming bacteria	20	Acid	Very active	Lactic acid	1st day	3 weeks
	40	Acid	Active	Lactic acid	2nd-3rd day	3 weeks
	60	Acid	Very slight			
Yeasts.....	20	Gaseous	Active	CO ₂ and alcohol	3rd-5th day	2 weeks
	40	Gaseous	Active	CO ₂ and alcohol	7th day	17 days
	60	Gaseous	Active	CO ₂ and alcohol	11th-12th day	17-21 days

* Brine temperature about 80 deg. F. during the fermentation period.

** After heading tanks.

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Discussion of Results

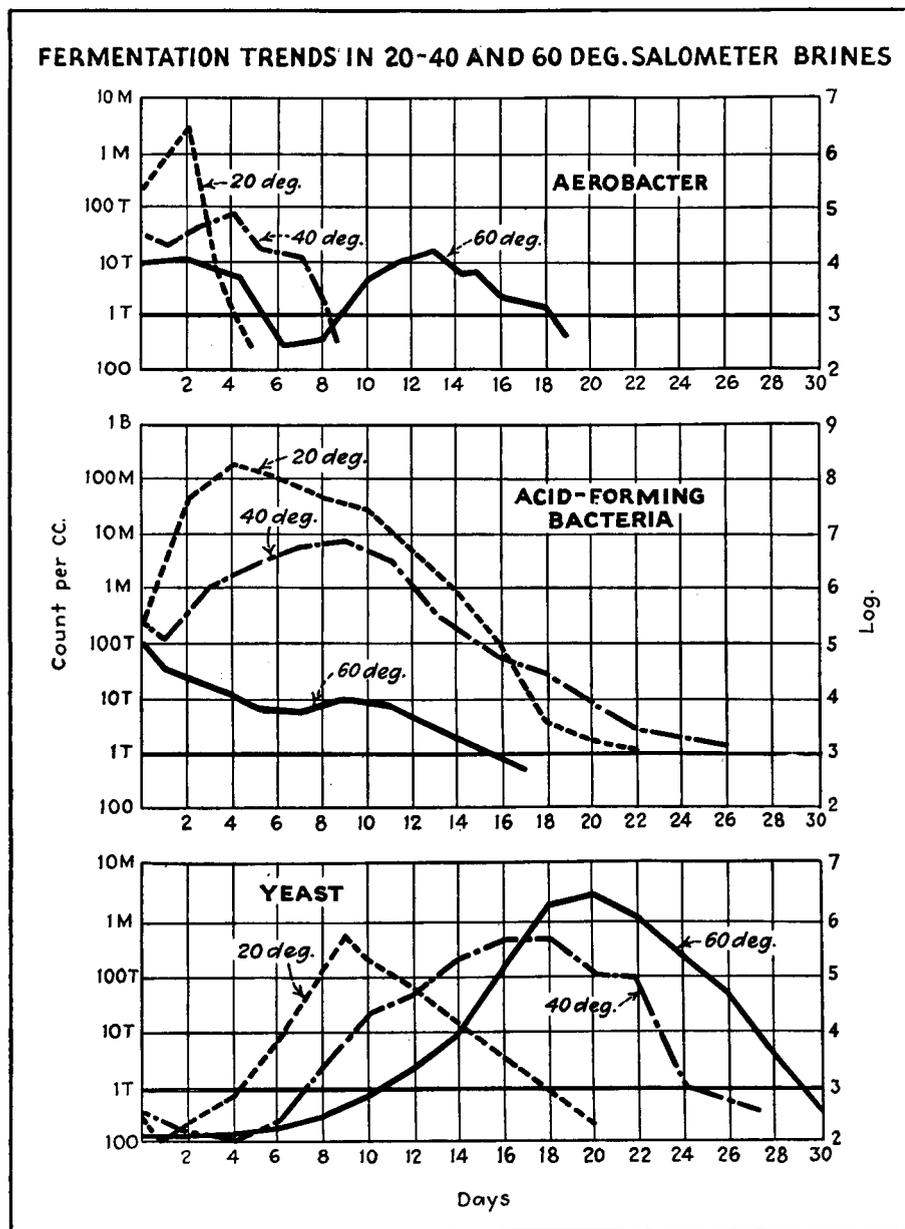
The values for the populations of all three types of organisms (Aerobacter, acid-forming bacteria and yeasts) shown in the accompanying chart are plotted logarithmically, principally to facilitate showing counts before and after active fermentation, as well as counts that vary greatly during the active fermentation. Counts less than 1,000 per cubic centimeter are shown below the double line drawn parallel to the abscissa, opposite 1T on the ordinate. Values less than 100 per cubic centimeter are not plotted. Although the fermentation trends for acid-forming bacteria and yeasts are based on duplicate fermentations for two or more seasons, for the Aerobacter group the data represent duplicate fermentations for one season only. This is due principally to the fact that this phase of the fermentation was only recently recognized. However, the results obtained to date give a reasonable indication of their activity in fermentations at the various salt concentrations.

The curves for the three brine concentrations show populations of the Aerobacter, acid-forming bacteria and yeasts at intervals of two days up to about 30 days.

In the 20-deg. brines (those with an initial concentration of 20 deg. sal. increased 10 deg. per week to 60 deg.) the population curve for the Aerobacter group shows that these organisms have a relatively short period of activity, since, after the first two-day interval there was a sharp decline to the 1,000 per cubic centimeter level by the fourth day. The population curve for the acid-forming bacteria rose sharply, so that a peak above the 100 million per cubic centimeter range was reached on the fourth day. The period of marked activity lasted for about two weeks, then the counts declined so as to be within the 1,000 per cubic centimeter range after about three weeks.

The fermentation trend shows that after a period of relative inactivity for about five days, a typical yeast fermentation occurred, reaching a peak on about the ninth day and then declining so as to reach the 1,000 per cubic centimeter range on about the 18th day. At this time active fermentation was considered to be ended.

With the brines having an initial concentration of 40 deg. sal. (increased 5 deg. per week to 60 deg.) fermentation trends are somewhat different from those for a 20-deg. initial concentration. In the case of the population curve for Aerobacter, it is demonstrated that the fermentation started promptly and covered a relatively active period of about



Growth of predominating microorganisms in cucumber fermentations at initial brine concentrations of 20, 40 and 60 deg. sal. (percent saturation with respect to salt). The 20-deg. brine was increased 10 deg. per week to 60 deg. and the 40 deg. brine was increased 5 deg. per week to 60 deg.

five days, during which time the counts were above the 10,000 per cubic centimeter range. After this time there was a sharp decline to the 1,000 per cubic centimeter range on about the eighth day.

The period of active Aerobacter fermentation corresponds very well with the time required for the acid-forming bacteria to reach the peak of their fermentation. In this instance, the population curve for acid-formers indicates a rather gradual rise to a peak close to the 10 million per cubic centimeter range on about the eighth day. After this the acid fermentation subsided somewhat gradually, so as to be practically ended after about three weeks.

The yeast fermentation trend shows these organisms initially present in the brine in numbers less than 1,000 per cubic centimeter and remaining in that range for about a week. After this period the counts rose so as to reach a peak well above the 100,000 per cubic centimeter range during the period between the 16th and 18th day. After this the fermentation subsided so that it was practically ended after about 24 days.

The influence of high-salt brines (60-deg.) shows very interesting relationships with regard to the growth of the types of organisms under discussion. The number of Aerobacter present in the brine constantly declined during

the first six days. Then, after remaining more or less inactive for a short interval, a fermentation by these organisms ensued, covering an active period from about the 11th to 18th day.

The acid-forming bacteria were markedly inhibited at this salt concentration, as demonstrated by the progressive decline in populations during the examination period.

The yeast fermentation trend shows the effect of salt in the form of an interval of inactivity for about 11 days, during which yeasts were present in the brine in numbers less than 1,000 per cubic centimeter. After this period the counts rose so as to reach a peak above the one million per cubic centimeter range on about the 21st day, then declined so the fermentation was over after approximately one month.

Influencing Factors

Influencing factors that are apparent from the results presented in the curves and other considerations with regard to the three fermentations, based on observations over a period of several seasons, will be discussed.

The 60-deg. treatment probably shows the most consistent behavior with reference to activity of the Aerobacter, resulting in what might be termed a typical hydrogen fermentation. A vigorous hydrogen fermentation is usually encountered. Likewise, with the 40-deg. treatment the typical hydrogen fermentation is common, although frequently with this salting treatment the hydrogen fermentation will take place with only moderate activity. On the other hand, with the 20-deg. treatment the vigorous, prolonged formation of hydrogen generally does not occur. Hydrogen may be detected in the fermentation gases, but only in very low concentration.

The rapid onset of acid fermentation in 20-deg. brines cuts short the activity

of the Aerobacter group because these organisms are unable to tolerate the increasing acid content of the brine. This accounts for the presence or absence of hydrogen in fermentations at this concentration. The same explanation will serve in part for the behavior of 40-deg. brines. Here active Aerobacter fermentation appears to be somewhat restricted to brines wherein delayed acid fermentation is encountered or where the predominating acid-forming bacteria are weak acid producers. The initial inoculum provided by the cucumbers in all probability plays a part in directing the course of fermentation.

The influence of salt alone is most clearly demonstrated with respect to acid-forming bacteria. There is a direct correlation between the populations of these organisms and the brine concentration used. Highest populations occur in the 20-deg. brines and correspondingly lower populations at increasing brine concentrations. Also, the fermentation in 20-deg. brines starts earlier. It appears that the activity of the acid-forming bacteria is terminated largely as the result of the combined effect of the brine salt concentration and the concentration of acid developed.

It has been previously demonstrated⁵ that the addition of sugar to brines near the conclusion of the acid fermentation brought about no change in the constant decline in the numbers of acid-forming bacteria present. However, if sugar was added at the beginning or during active fermentation, a significant increase in the number of acid-forming bacteria was brought about. This increase in population was not reflected in an increase in acid production.

Comparison of yeast fermentation in the three brines shows that there is a direct correlation between the salt concentration used and the onset of active fermentation. The starting intervals required were about 4, 7, and 11 days, respectively. The duration of the fermentation in 20-deg. brines was somewhat shorter than in the case of 40-deg. brines, although there was no apparent difference in maximum yeast populations once fermentation was under way.

Appearance of a typical vat during the phase of fermentation characterized by a vigorous evolution of gaseous products.

In general the results indicate that yeast fermentation in 60-deg. brines reaches higher maximum numbers than in either of the lower concentrations. The activity of the yeasts is apparently terminated when the sugar in the brine is reduced to a very low concentration or completely utilized. In the experiments involving addition of sugar to brine,⁵ the addition of sugar resulted in a marked increase in the yeast population and in the rate of gas evolution.

Some general relationships regarding all the fermentations previously discussed are summarized in the accompanying table. Fermentations with Aerobacter and with yeasts are generally associated with bloater production. Fermentation with the acid-forming bacteria is characterized by preservation of the pickles. This preservative fermentation is active only in the 20- and 40-deg. brines, activity being very slight in 60-deg. brines.

Summary

In general, the bacteriological findings demonstrated that active yeast fermentations were found in all brine treatments used. Active fermentations by the acid-forming bacteria were restricted to the 20- and 40-deg. brines. General observations revealed that an active hydrogen fermentation by the Aerobacter group was usually encountered in 60-deg. brines, while in the 40-deg. brines an active hydrogen fermentation may or may not take place. A relatively short fermentation by this group of organisms may occur in 20-deg. brines or it may be absent.

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