[Reprinted from FOOD RESEARCH, 1939, Vol. 4, No. 6]

# GASEOUS PRODUCTS OF CUCUMBER PICKLE FERMENTATIONS[1,2]

M. K. VELDHUIS AND JOHN L. ETCHELLS
*Bureau of Chemistry and Soils, U. S. Department of Agriculture, Raleigh, North Carolina*[3]

(Received for publication, May 31, 1939)

The normal fermentation of cucumber pickles in salt brines is accompanied by the evolution of considerable quantities of gases. At times during the production of gases, a froth may be formed on the surface of the brine to a depth of as much as four or five inches. The presence of carbon dioxide in these gases has been mentioned in the literature, but no systematic study of them has been reported. A study of the gases evolved was undertaken at a temporary field laboratory located at a commercial cucumber pickling plant at Faison, North Carolina. At this plant facilities were provided for the experimental salting of cucumbers using brines of various salt concentrations; 85-bushel vats were used for the purpose.

The evolution of gases from vats has been taken more or less for granted by commercial cucumber salters. It has been used as an index to the rate of fermentation, and when exceptionally large amounts of gas were evolved, the salt concentration of the brine has been increased to decrease the rate of fermentation. When these studies were first undertaken, analyses for carbon dioxide and oxygen were made and it was found that in some cases during active fermentation the carbon dioxide content of the gases was above 99 per cent. It soon became apparent, however, that at times some other gas besides carbon dioxide was being formed because some samples collected during rapid evolution of gases contained less than 50 per cent carbon dioxide and no oxygen. It was then found that the bubbles on the surface of some brines would explode with a distinct pop when touched with a lighted match. Changes were made in the gas-analysis outfit so that combustible gases could be determined. The combustible gas was found to be hydrogen, but it was also found that hydrogen was not produced by all the cucumber-pickle fermentations. The effect of various factors on the production

[1] Presented at the 97th meeting of the American Chemical Society, Baltimore, Maryland, April 3-7, 1939.

[2] This work was done as a part of a coöperative project between the Bureau of Chemistry and Soils, and the Department of Horticulture, North Carolina Agricultural Experiment Station.

[3] Food Research Division contribution No. 423.

of carbon dioxide and hydrogen, and a study of the microörganisms responsible for the production of these gases proved interesting.

## ANALYTICAL METHODS

The gases were analyzed in an apparatus similar to the Williams gas analysis outfit, Model B. Carbon dioxide was determined by absorption in a concentrated solution of potassium hydroxide and noting of the decrease in volume. Oxygen was determined by absorption in alkaline pyrogallol solution. Hydrogen was determined by burning with added oxygen in an electric spark explosion burette and noting the decrease in volume. The absence of methane or other combustible hydrocarbon gases was shown by passing the products of combustion through the potassium hydroxide to absorb any carbon dioxide and noting no decrease in volume. Adjustments in the oxygen-sample ratio nearly always resulted in an explosion. Amounts of hydrogen as low as .3 and .2 per cent were determined by this method.

## PRELIMINARY EXPERIMENTS

Cucumbers have small bubbles of gas included in their structure which give a rather opaque, white appearance to the sliced fruit. As the cucumbers cure in brine these bubbles disappear and leave the tissues somewhat translucent. These bubbles may be removed by the repeated application of vacuum or by heating. In order to obtain an approximation of the amount and composition of this gas, a flask was filled with cut cucumbers, water was added, and the water boiled slowly. The gases driven off were collected over saturated brine, measured, and analyzed. A kilogram of cucumbers treated in this manner yielded 94 cubic centimeters of gas of which 37.6 cubic centimeters were carbon dioxide and 1.4 cubic centimeters were oxygen. An analysis for hydrogen was not made, but the remainder was undoubtedly nitrogen. This method may be subject to some criticism, but at least it gives an idea of the extent and composition of the gas in fresh cucumbers. These gases, from the interiors of the cucumbers seem to come out at a fairly constant rate during the fermentation; and nitrogen from this source probably accounts for the sum of the carbon dioxide, oxygen, and hydrogen falling short of 100 per cent, especially during slow evolution of gases.

Although no attempt was made to measure the quantity of gas evolved during the fermentations in vats, a laboratory experiment gives some idea as to the amount of gas evolved. A three-liter flask was packed with cucumbers and allowed to ferment in a brine of 15 per cent saturation for four days and then salt was added to bring the salt content to 30 per cent saturation. During the fermentation

4,800 cubic centimeters of gas were collected, of which 4,600 cubic centimeters were carbon dioxide. No analyses for hydrogen were made. In this case the volume of the gas was slightly over one and one-half times the volume of the fermenting brine plus cucumbers. This treatment corresponds roughly to the 20° salometer treatment explained later. It was observed that vat fermentations in 40 and 60 per cent saturated brines gave off considerably larger volumes of gas than fermentations in brines of 20 and 30 per cent saturation; and since there were variations with the same treatment, this can be considered only a very rough estimate.

## EXPERIMENTAL PROCEDURE

In general, commercial practices were followed in salting the cucumbers in the experimental vats. The vats were filled with cucumbers, a layer of boards was fastened in place over the cucumbers, and salt brine added to a level about four inches above the layer of boards. Brines of 20, 30, 40, 60, and 80° salometer were used to cover the cucumbers. The degrees salometer of a brine indicates the percentage of saturation of the brine with respect to salt. Thus, a 100° salometer brine would be saturated and contain 26.4 per cent sodium chloride. Additions of salt were made to the vats to maintain the brines at the indicated salometers during the first week, and then salt was added to the 20, 30, and 40° salometer brines so as to raise the concentrations to 70° salometer by the end of the sixth week. The 60° brine was raised to 70° salometer during the sixth week. The 80° salometer brine was maintained at that concentration. The salting procedures will be indicated by the initial brine concentrations. The temperatures of the brines during the fermentations were within a few degrees of 26.7°C.(80°F.). Most of the vats were outside and unsheltered. Three sheltered vats will be indicated. No special starter or inoculation was used. The cucumbers were not washed and undoubtedly an abundance of microörganisms of various types were introduced by the cucumbers and adhering particles of soil. Inverted funnels were placed over cracks in the layer of boards that held the cucumbers immersed in the brine, and samples of the evolving gases collected in the funnels by displacement. The samples were transferred to the gas-analysis outfit for analysis.

A determination for oxygen was made on all samples, but the values are omitted from the tables. The values were too small to be of significance. During the rapid evolution of gases little or no oxygen was detected. The amounts ranged from none to two or three tenths of one per cent. It is believed that the oxygen came largely from the small bubbles in the interiors of the cucumbers.

Larger amounts of oxygen were an indication that something was wrong with the sample, and the oxygen determination served as a check to indicate whether any air had been introduced into the samples.

## RESULTS OF GAS ANALYSES

Some of the results of the gas analyses with the 60° salometer brine fermentations are given (Table 1). The gas collected on the second day probably contained a large amount that had been trapped in the vat during filling and also some gas coming from the interiors of the cucumbers, as indicated previously. From the second to the

TABLE 1

*Analyses of Gases Produced by Fermentation of Cucumbers in 60° Salometer Brines*

| Age | Vat 7 | | Vat 11 | |
|---|---|---|---|---|
| | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ |
| *days* | *pct.* | *pct.* | *pct.* | *pct.* |
| 2 | 45.0 | 0.0 | 38.7 | 0.0 |
| 12 | 33.9 | 56.2 | 17.8 | 35.4 |
| 15 | 60.4 | 39.1 | 41.4 | 35.8 |
| 19 | 52.3 | 40.4 | 51.5 | 40.8 |
| 23 | 65.0 | 9.1 | 72.8 | 20.5 |
| 27 | 87.4 | 10.0 | 79.8 | 15.1 |
| 31 | 88.7 | 8.8 | 93.7 | 3.9 |
| 37 | 90.2 | 8.5 | 89.2 | 7.1 |
| 41 | 78.8 | 4.1 | 81.4 | 12.2 |
| 45 | 68.7 | 24.4 | 82.7 | 9.3 |
| 50 | 70.5 | 18.0 | 90.7 | 4.4 |

12th day the evolution of gas was so slow that there was an insufficient amount for analysis. On about the 12th day the evolution of gas started and continued until after the 50th day, when the analyses were discontinued. The most important result to be noted is the persistence of a relatively high percentage of hydrogen throughout the fermentations. During the early part of the active fermentation the content of hydrogen ranged around 40 per cent. On about the 21st day the percentage of hydrogen began to decrease.

Some of the analyses of gases from fermentations in 40° salometer brines, with the different treatments indicated (Table 2), show that hydrogen was found in only one vat, No. 14, and it was found as long as the evolution of gases continued from that vat. Even in the case of this vat the percentage of hydrogen was much less than in the cases with 60° salometer brines. The brines in Vats 6 and 10 were circulated for an hour a day with a pump and it was noted that gases were given off over a shorter period of time from these

TABLE 2

*Analyses of Gases Produced by Fermentation of Cucumbers in 40° Salometer Brines*

| Age | Vat 1 Regular | | Vat 14 Regular | | Vat 2 Sheltered | | Vat 5 Sheltered, stirred | | Vat 6 Sheltered, circulated | | Vat 10 Circulated | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ |
| *days* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* |
| 2 | 56.3 | 0 | 46.9 | 0.0 | 49.1 | 0 | 43.0 | 0 | 39.2 | 0 | 44.2 | 0 |
| 7 | 66.8 | 0 | 54.1 | 21.6 | 70.7 | 0 | 54.8 | 0 | 51.1 | 0 | 56.4 | 0 |
| 10 | 53.0 | 0 | 60.7 | 14.0 | 35.6 | 0 | 59.7 | 0 | 57.5 | 0 | 38.8 | 0 |
| 14 | 91.0 | 0 | 91.0 | 0.3 | 61.3 | 0 | 43.5 | 0 | 52.0 | 0 | 91.6 | 0 |
| 20 | 85.4 | 0 | 82.4 | 1.7 | 80.7 | 0 | 89.9 | 0 | 89.0 | 0 | 88.2 [1] | 0 [1] |
| 24 | 87.0 | 0 | 92.2 | 1.1 | 76.2 | 0 | 90.7 | 0 | ..... | .... | ..... | .... |

[1] Age 17 days.

vats than from uncirculated vats with the same brine concentration. Gas evolution continued until about the 30th day from the four uncirculated vats as compared with more than 50 days from 60° salometer brines.

Analyses of gases from fermentations in 20 and 30° brines indicate that both fermentations in 30° brines produced hydrogen and only one in the 20° brines (Table 3). The amount of hydrogen produced in one 30° fermentation, vat No. 9, was very small. In no case was the production of hydrogen as great as that in 60° salometer brines. The production of gases from these vats was less and extended over a shorter period of time than that from vats containing

TABLE 3

*Analyses of Gases Produced by Fermentation of Cucumbers in 20 and 30° Salometer Brines*

| Age | 20° Salometer brine | | | | 30° Salometer brine | | | |
|---|---|---|---|---|---|---|---|---|
| | Vat 12 | | Vat 8 | | Vat 9 | | Vat 13 | |
| | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ |
| *days* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* | *pct.* |
| 2 | 63.1 | 0.0 | 63.6 | 0 | 63.1 | 0.0 | 47.4 | 5.1 |
| 5 | 75.0 | 0.0 | 69.5 | 0 | 71.4 | 0.0 | 51.8 | 20.0 |
| 8 | 43.2 | 1.4 | 52.8 | 0 | 61.8 | 0.0 | 35.3 | 7.6 |
| 11 | 71.6 | 0.7 | 70.2 | 0 | ..... | ..... | ..... | ..... |
| 15 | 69.2 | 0.6 | 55.1 | 0 | 76.5 | 0.3 | 60.3 | 9.4 |
| 17 | 59.4 | 0.5 | ..... | .... | 84.3 | 0.1 | 72.2 | 5.7 |
| 19 | ..... | ..... | 53.4 | 0 | 89.4 | 0.0 | 75.8 | 1.9 |
| 23 | 43.9 | 0.0 | ..... | .... | ..... | ..... | 66.7 [1] | 2.1 [1] |
| 27 | ..... | ..... | ..... | .... | 81.6 | 0.0 | 60.7 [2] | 1.3 [2] |

[1] Age 22 days. [2] Age 26 days.

40 and 60° salometer brines. It was noted that the sum of the percentages of carbon dioxide and hydrogen, or the percentage of carbon dioxide alone in cases where no hydrogen was produced, lacked considerable of making 100 per cent. This was probably due to nitrogen from the interiors of the cucumbers making up a large percentage of the gas samples during the slow evolution of fermentation gases.

Several analyses from additional vats (Table 4) serve to emphasize the preceding data. Single analyses from 11 additional 40° salometer vat fermentations are shown. Of these only three showed the presence of hydrogen. R8V6, a regular commercial vat, was interesting. It was gassing at an exceptionally rapid rate and attention was called to it by the plant manager. When a lighted match was applied to the surface bubbles, the bubbles exploded vigorously.

The two analyses of the gases from fermentations in 60° salometer brines show considerable quantities of hydrogen in keeping with the

results shown above. The analysis of the gas from an 80° salometer fermentation is included. The evolution of gas was very slow from this vat; the sample of gas was small and collected over several days' time. Nevertheless, it shows the presence of a considerable proportion of hydrogen.

Analyses were also made of the gas from hollow cucumber pickles. These hollow cucumbers are commonly called "bloaters" by the trade and are formed during the fermentation. Gases appear in the cucumbers and the three carpels separate in the interior. The separation does not extend to the outside of the cucumbers. The gas-filled cavity may extend until it comprises as much as three-fourths of the volume of the cucumber, and at times the fruits are under tension owing to gas pressure from within. This condition occurs more frequently with the large-sized cucumbers. When the cucumbers are allowed to remain in the brine for some time, brine gradually replaces the gas.

TABLE 4

*Analyses of Gases From Fermentation of Cucumbers in Brines of Various Concentrations*

| Vat No. | Age | Brine concentration | Gas composition | |
|---|---|---|---|---|
| | | | $CO_2$ | $H_2$ |
| | *days* | *° sal.* | *pct.* | *pct.* |
| 193 | 12 | 40 | 95.0 | 0.0 |
| 178 | 12 | 40 | 58.5 | 0.0 |
| 191 | 12 | 40 | 83.5 | 0.0 |
| 187 | 12 | 40 | 79.4 | 6.5 |
| 4-37 | 16 | 40 | 93.1 | 0.0 |
| 6-37 | 22 | 40 | 90.9 | 0.0 |
| 7-37 | 12 | 40 | 90.5 | 0.0 |
| 9-37 | 23 | 40 | 86.2 | 0.0 |
| 10-37 | 15 | 40 | 97.5 | 0.0 |
| R8V6 | 2 | 40 | 59.0 | 37.2 |
| R16V6 | 10 | 40 | 99.4 | 0.2 |
| 1-37 | 21 | 60 | 63.8 | 30.3 |
| 5-37 | 16 | 60 | 31.6 | 55.5 |
| 8-37 | 28 | 80 | 21.4 | 33.3 |

The analyses of gases from "bloaters" compared with analyses of gases collected at the top of the vats at the same time show a marked similarity in compositions (Table 5). When hydrogen was found in surface gas, it was also found in the "bloater" gas in approximately the same proportion. The percentages of carbon dioxide were also found to agree quite well. Undoubtedly these gases were formed within the cucumbers. However, since "bloaters" were

found in vats from which there was no evolution of hydrogen as well as in vats from which hydrogen was evolved, a fermentation-forming hydrogen within the cucumbers cannot be considered the sole cause of "bloater" formations.

It was also shown that gases were produced in the body of the brine itself. Some brine from an active fermentation-producing hydrogen and carbon dioxide was drawn off into a flask and allowed to ferment. The gas produced in this flask was similar in composition to that collected in the usual manner. It seems that the gases are produced in both the interiors of the cucumbers and in the brine, and that the gases from both sources are similar in composition.

TABLE 5

*Analyses of Gases From Hollow Cucumbers Compared With Analyses of Gases Collected in the Usual Manner*

| Vat No. | Age | Brine concentration | Gas from hollow cucumbers | | Corresponding surface gas | |
|---|---|---|---|---|---|---|
| | | | $CO_2$ | $H_2$ | $CO_2$ | $H_2$ |
| | *days* | *° sal.* | *pct.* | *pct.* | *pct.* | *pct.* |
| 3-37 | 17 | 20 | 31.4 | 0.0 | 25.9 | 0.0 |
| 13-38 | 18 | 30 | 83.2 | 0.6 | 75.8 | 1.9 |
| 4-37 | 17 | 40 | 91.4 | 0.0 | 93.1 | 0.0 |
| 6-37 | 22 | 40 | 92.0 | 0.0 | 90.9 | 0.0 |
| 1-38 | 18 | 40 | 90.6 | 0.0 | 89.2 | 0.0 |
| 14-38 | 18 | 40 | 91.4 | 0.7 | 89.0 | 1.6 |
| 1-37 | 24 | 60 | 35.6 | 29.9 | 60.8 | 19.2 |
| 1-37 | 24 | 60 | 61.8 | 31.4 | 60.8 | 19.2 |
| 5-37 | 16 | 60 | 48.7 | 44.7 | 31.6 | 55.5 |
| 7-38 | 16 | 60 | 46.0 | 42.7 | 52.3 | 40.4 |

## BACTERIOLOGICAL STUDIES

Bacteriological studies during the past three years have shown a definite correlation between the carbon dioxide formation during the latter part of the active fermentation and the presence of a typical yeast fermentation. This was especially true of the fermentations in 40° salometer brines, in which typical yeast fermentations started on about the 10th day and continued for about one week. During this period yeast counts of 10,000 to 100,000 per cubic centimeter were obtained.

Bacteriological studies were undertaken to ascertain if microörganisms were responsible for the production of hydrogen. Vat R8V6, mentioned previously as producing large amounts of hydrogen, was thought to present an ideal opportunity for this study. This vat was gassing rapidly during the initial stage of the fermentation. Bacteriological plate counts were made and these indicated the following

numbers of organisms per cubic centimeter: 22 million acid-formers, 200 thousand peptonizers (soil group), and 200 yeasts. Numerous isolations of typical colonies of acid-formers were made and transferred into fermentation tubes using cucumber-juice broth as the liquid medium. None of the resulting fermentations showed the production of gas. It was assumed that the predominating organisms, those occurring in millions per cubic centimeter, were not responsible for the hydrogen production. The ordinary routine plating methods as used for following the brine fermentations were not suitable for the isolation of hydrogen-producing organisms.

Later on other vats were used in a more intensive effort to cultivate the microörganisms responsible for the hydrogen production. This involved numerous platings and the employment of various types of solid media both aerobically and anaerobically. Finally it was demonstrated that the causitive agent could be isolated from the brine by culturing under strict anaerobic conditions. Plate counts indicated that these organisms were present in low numbers as compared with the total count. Cultures isolated and inoculated into Smith fermentation tubes using cucumber-juice broth showed rapid gas production in 48 hours. This gas was analyzed and found to contain a relatively high percentage of hydrogen. These organisms are being studied further in regard to their cultural characteristics and identification.

It should be noted that this work was done under southern conditions. The fermentations in vats took place largely during the month of July at temperatures close to 26.7°C. (80°F.). In the northern part of the United States, such as in the Great Lakes region, the season is much later. The crop there is harvested in August and September and the fermentations occur largely during the relatively cool months of September and October. Somewhat different methods of brining are used in these regions. Usually the strength of the brine is not increased as rapidly as in southern sections. Changes in conditions like these might have considerable effect on the gas production by the fermentation.

## SUMMARY

Carbon dioxide was formed during the fermentation of cucumber pickles in brines ranging from 20 to 80 per cent saturation with respect to salt.

Larger quantities of gases were evolved during fermentations in 40 and 60 per cent saturated brines than in brines of 20, 30, and 80 per cent saturation.

Hydrogen was produced in considerable amounts by all the fermentations observed in 60 per cent saturated brines. Hydrogen was produced in smaller amounts by some fermentations in 20, 30, and 40 per cent saturated brines; however, hydrogen was not produced by all fermentations at these brine concentrations.

Organisms capable of producing hydrogen have been isolated from brines.

Gases formed in the interiors of hollow cucumbers or "bloaters" had approximately the same composition as gases collected at the tops of vats from which they were taken.