

The Preservation of Brine Samples for Chemical Analysis¹

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IN doing experimental work on the manufacture of cucumber pickles it was found advisable to have a method for keeping samples of brine so that they could be set aside and the chemical analysis made at a later date. This was particularly true of the green cucumber season when samples could not be analyzed as soon as taken. Tests with several preservatives were conducted with this in mind. These tests should be of interest especially to salters who make chemical analyses or who might want to send samples to their central laboratories for analysis.

The main qualities desired in a preservative were that it prevent any chemical change or bacteriological action from the time of taking of the sample until the analysis of the sample was complete, that it not interfere with any of the analyses to be made, and that it be of such strength that only a small quantity need be added.

Several chemicals which seemed to offer possibilities were given a preliminary trial in brine in test tubes to determine which would effectively reduce the number of bacteria present. Compound I (sodium orthophenylphenolate), compound II (sodium 2, 4, 5, trichlorophenolate), chloroform, toluene, thymol, formaldehyde, copper sulphate, and phenol were tried. Of these, formaldehyde, copper sulphate, and phenol were found to be definitely unsuitable. For the main experiment compound I, compound II, compound III (sodium 2 chlor 6 phenylphenolate), compound IV (sodium 2, 4, 5, 6 tetrachlorophenolate)³, chloroform, toluene, and thymol were chosen.

Six brines were prepared for the tests. They were a 20° (20 per cent saturated) brine a few days old, a 60° brine a few days old, a 60° brine about six months, and duplicates of the above three brines with added dextrose and lactic acid. The addition of dextrose was to provide brines with ample food to support bacterial growth and the lactic acid was added to provide brines of higher acidity. The titratable acidities of the brines ranged from 0.04 to 0.70 per cent calculated as lactic acid. The reducing sugars ranged from 0.04 to 0.33 per cent calcu-

lated as dextrose. The pH values ranged from 2.95 to 5.85.

Portions of the brines were placed in one quart jars. Compounds I, III, and IV were added to respective jars in the proportion of 1-5,000, compound II in the proportion of 1-10,000, and chloroform, toluene, and thymol were added to respective jars until the brines were saturated and an excess present. Jars in duplicate of each of the six brines were put down with each chemical. Jars of brine were also placed in the refrigerator and at room temperature for comparison.

A count of the number of bacteria present in the six initial brines and in the brine in each jar after storage of one and five months was made. At the same time the content of reducing sugar, the titratable acidity, and pH of each brine were determined. Reducing sugars were determined by the Shaffer and Hartmann micro method standardized to the conditions under which it was used. The titratable acidities were determined by titration with standard sodium hydroxide using phenolphthalein as the indicator. A quinhydrone electrode was used to determine pH.

In evaluating the data differences of one-twentieth or less from the original reducing sugar or titratable acidity content or variations in pH values of less than 0.05 pH were not considered to be significant.

Compounds I, II, III, and IV gave quite satisfactory results. None interfered with the chemical analyses being made, and there were no significant changes in sugar content, pH, or titratable acidity during storage. A very slight rise in the acid content during five months' storage was noticed, but this was not large enough to be of any importance. The count of bacteria was reduced from millions per cc. at the start to around a hundred per cc. after five months' storage. It seemed that compound II was a little the better and it has been used extensively since then for preserving samples. Compounds I and III produced a brown discoloration in one brine.

SAMPLES preserved with chloroform had the lowest count of bacteria at the end of one and five months' storage. There was no noticeable change in pH or titratable acidity during storage. Unfortunately, chloroform

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³ These compounds were furnished by the Dow Chemical Company of Midland, Michigan.

interfered with the determination of reducing sugars. Perhaps a satisfactory method for the removal of the chloroform before the sugar analysis is made could be worked out, but it would involve an extra step in the procedure. Chloroform is regarded as being satisfactory when one is interested in titratable acidity and pH.

Toluene did not interfere with the chemical analyses and was about as efficient as compounds I, II, III, and IV in the reduction of the number of bacteria during the first month. There was a very slight decrease in the sugar content and almost no change in titratable acidity or pH during this period. At the end of five months the results were entirely different. The bacterial count in some jars was in the tens of thousands per cc., a large proportion of the sugar in the samples had been destroyed, and some samples showed a considerable increase in acid content. Toluene floats on the top of brine and since the jars were not absolutely airtight, some of it undoubtedly evaporated. Perhaps toluene would be better in sealed containers but all the samples removed from the brine would have to be taken through a layer of toluene. This would be particularly troublesome when it is desirable to pour out a small portion as in the determination of pH.

Samples in which thymol was used as a preservative did not keep very well even though the bacterial count was reduced to about a thousand per cc. at the end of a month and to about a hundred per cc. at the end of five months. There was an evident decrease in the sugar content at the end of one month and even more at the end of five months. In some cases there was a decrease in the titratable acidity and in others there was an increase. There was no appreciable change in pH.

BRINES placed in the refrigerator did not keep any too well. There was a reduction in the bacterial count but it was still in the hundreds of thousands per cc. at the end of one month and in the tens of thousands at the end of five months. Of course the idea was to keep the samples in this case by stopping bacterial action rather than by killing the organisms present. There was a decrease in the sugar content during the first month of storage and a further decrease during five months. Most of the brines showed a slight increase in titratable acidity and no noticeable change in pH during the storage.

Quite naturally brines at room temperature did not

keep well. Those with the low salometer reading changed the more rapidly. In most cases there was an increase in titratable acidity during storage, but in one case it decreased. As was to be expected there was not very much change in the old brine without added sugar or acid. There should be no difficulty in keeping old brines that have fully fermented out for short periods of time provided they are kept in well filled closed containers.

Upon looking over the data it was decided that compound II was the more satisfactory for brine samples when the amount of reducing sugar as well as the titratable acidity and pH were to be determined and that either compound II or chloroform was satisfactory when the determination of reducing sugar was to be omitted. Several hundred samples have been preserved with compound II and there has been no evidence that any of the samples have failed to keep.

Compound II is soluble in water and in use a 10 per cent solution of it is prepared. A half cubic centimeter added to 500 cc. of brine (about 10 drops to a pint) gives a concentration of 1-10,000, the concentration used. Pint bottles with crown caps such as beer or soft drink bottles have been found to be very satisfactory for keeping samples by this method.

Chloroform has also been used extensively to preserve samples for titratable acidity and pH determinations only. It has the advantage that it can be obtained from any drug store. Enough of it should be used to saturate the brine and also provide an excess which sinks to the bottom. Whether the sample is preserved with chloroform or compound II, it should be shaken to distribute the chemical. About 5 cc. (one teaspoonful) per pint of brine is sufficient. Chloroform is quite volatile and in order to prevent its evaporation, the container should be tightly sealed or capped. Screw top pickle jars have not been found to be entirely satisfactory.

Samples preserved with these materials are valueless for bacteriological analysis and the chemicals used in these tests render materials unfit for human consumption.

Mr. John L. Etchells of the Bureau of Chemistry and Soils made the bacteriological plate counts in the above tests. Dr. L. H. James, who was with the Bureau at the time these tests were made, was helpful in outlining the experiments and Dr. Ivan D. Jones of the North Carolina Experiment Station was also of assistance in getting the experiments under way.