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A QUICK TEST FOR CUCUMBER BRINE ACIDITY

SUMMARY—A quick, simple test has been developed for the pickle industry to determine brine acidity of 0.60% and above as lactic acid in tanks of fermenting cucumbers. Tris (hydroxymethyl) aminomethane was used as an acidimetric standard and was impregnated together with phenol red as an indicator in filter paper. 1.25-in. squares of the paper were developed to neutralize 5.0 ml of 0.60% lactic acid at pH 5.8. The paper squares have been successfully used by members of the pickle industry to test their fermenting pickle brines under actual commercial conditions.

INTRODUCTION

IN THE FERMENTATION of cucumbers under commercial conditions, the development of a suitable acid concentration is important to final quality and preservation of the brine-stock pickles. The rate and amount of acid production, usually expressed as lactic, is limited by such variables as cucumber size and variety, initial salt (NaCl) concentration of the cover brine, brine temperature and the natural microbial flora present in the brine (Etchells and Jones, 1946; Etchells et al., 1951; 1958; Jones, 1940). With the onset of brining a fall crop of cucumbers, in addition to the usual mid-summer harvest, it has become increasingly important to observe the rate of acid production in each fermentation because of the effect of the lower ambient temperatures that retard growth of the lactic acid bacteria which results in slower acid formation. Thus, it is imperative that the brine acidity should reach an acceptable level before the brine strength is increased. If the brine strength is increased according to an inflexible schedule, then the salt concentration may go beyond the active growth range for the lactic acid bacteria.

The amount of brine acid for a cucumber fermentation to develop during the first 5 to 10 days has been suggested (Etchells and Moore, 1971, unpublished, and private communication from F. O. Brown, Chicago, Ill., to J. L. Etchells) to be 0.6% (as lactic). When the acidity reaches this level, the salt concentration is increased. If each cucumber tank is to be checked daily, a rapid and fairly accurate brine acidity test would be needed by the plant operator. The usual method (Etchells et al., 1958) of testing the fermenting brine for total acidity requires the collection of a representative sample in a test tube or bottle, and unless run at once, the sample must be preserved in some manner; Dowicide B, toluene or Merthiolate® have all been used in our laboratory. A 10-ml sample is titrated with 0.111 N NaOH to a faint pink end-point with phenolphthalein as the indicator. This method is accurate, yet it requires time, equipment and a quality control laboratory.

The objective of this study was to develop a simple, rapid acid test for 0.6% lactic which could be made directly at the cucumber tank, if desired.

MATERIALS & METHODS

Standard solutions

Sodium hydroxide (0.111 N) was prepared according to the AOAC (1965) method with potassium hydrogen phthalate as a standard. With the standardized NaOH solution, the following acid standard solutions were made: 0.1014 N lactic acid, 0.0667 N (0.60%) lactic acid, 0.1040 N acetic acid, 0.1033 N hydrochloric acid, 0.1007 N sulfuric acid. In addition, four base solutions were prepared and standardized to a strength where 1 ml was equivalent to 10 ml of 0.60% lactic acid [2.67% wt/v sodium hydroxide, 5.71% barium hydroxide, 3.53% sodium carbonate, 8.07% tris (hydroxymethyl) aminomethane]. Also, a 1.00 N tris (hydroxymethyl) aminomethane solution was prepared and standardized against the standard lactic acid solution.

Indicators

Phenolphthalein 0.1% was prepared in 95% ethyl alcohol. Phenol red 0.05%, brom-thymol blue 0.05% and brom-cresol green 0.05% were prepared in .02 N NaOH or directly in tris (hydroxymethyl) aminomethane.

Acid-test paper

Sheets of No. 1 Whatman chromatography paper (46 by 57 cm) were impregnated with aqueous solutions of tris (hydroxymethyl) aminomethane (Fisher Scientific, Certified Reagent T-395, assay 99.9% pure) by a dipping technique. Two methods were used: the first method consisted of dipping of one-half sheets (46 by 28.5 cm) of the paper into a rectangular glass baking dish containing the chemical until they were completely saturated, then draining for approximately 30 sec and hanging them vertically to dry. The second method used a stainless steel chromatography tray (25 in. long, 1.5 in. wide and 5/8-in. deep, the bottom of the tray was the shape of one-half circle) and a 5/16-in. diameter glass rod (24 in. long) bent 2 in. at right angles at each end. One-half sheets of the paper were placed between the rod and the tray and pulled from the container between the rod and the tray edge. It was determined experimentally that with the latter method, the chemical was more evenly impregnated, particularly so when dried on a horizontal surface of plastic-coated freezer paper (Poly-Wrap, St. Regis Paper Co., 230 Park Ave., New York).

Equivalence pH points

The potentiometric titration curves were made using a 50-ml burette and a Beckman Expandomatic pH meter attached to Sargent, Model SRL recorder containing a manual event-marker.

Brine samples

Commercial brine samples, collected from tanks during the early period of fermentation, were obtained from pickle companies by the method described by Etchells and Jones, 1946, and Etchells et al., 1958.

Cucumber brine testing

The test using paper squares which equalize 5 ml of 0.6% lactic acid has been given the name Q-BAT (quick-brine-acidity-test). Instructions for the test are as follows: 1) Fold a single test-paper into thirds. 2) Drop to the bottom of a clean test tube; preferably a tube about 16-mm diameter (100–150 mm in length). 3) Pipette a 5-ml brine sample into the tube containing the test-paper; the brine should come to the top of the paper. 4) Shake gently for 2–3 sec. After 2 or 3 min, the color of the brine sample and paper should be the same. When it appears certain there will be no further color change, record the results of the test into one of the three categories given below: 5) Color test: Red = negative; less than 0.50% lactic acid. Orange = about 0.55% lactic acid. Yellow = positive; 0.60% lactic acid and higher.

RESULTS & DISCUSSION

Preliminary tests

Three common chemicals, used frequently to neutralize acidity, were examined for their possible use in a quick brine acidity test. Standard solutions of NaOH, $Ba(OH)_2$ and Na_2CO_3 were calculated and measured to where 1 ml of each would neutralize 10 ml of 0.60% lactic acid. The three bases were also tested as dried solid material in the bottom of test tubes placed there accurately by oven drying of a known quantity of the standard base. The two hydroxides neutralized the lactic acid as expected, but the dried samples took 10 min or longer to completely equilibrate. The hygroscopic nature of the two hydroxides in the solid state, plus the fact that they both reacted slowly with CO_2, ruled them out. Even though Na_2CO_3 has been used as an excellent standard for strong mineral

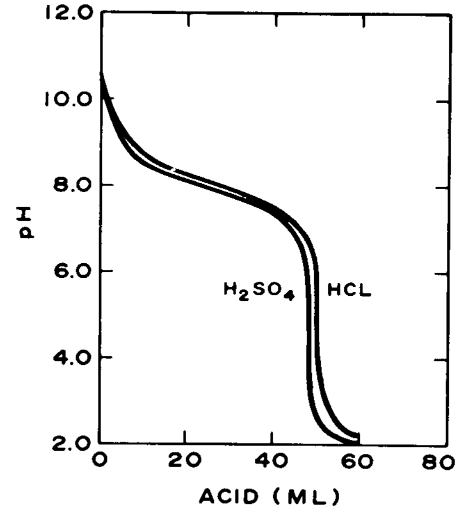

Fig. 1—Titration of tris (hydroxymethyl) aminomethane with 0.1 N HCl and with 0.1 N H_2SO_4. Equivalence points for HCl at pH 4.6 and for H_2SO_4 at 4.7.

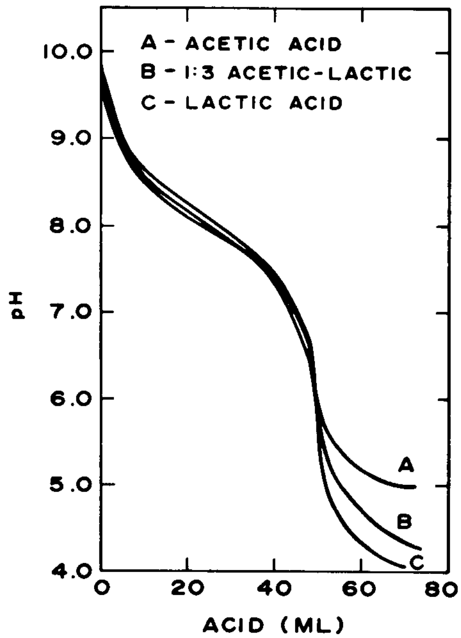

Fig. 2—Titration of tris (hydroxymethyl) aminomethane with 0.1 N acetic, 0.1 N lactic and a mixture of two acids.

acids, it was not suitable for weak organic acids, such as lactic. The buffering action of Na_2CO_3 to $NaHCO_3$ with a pK 10.4, and then to H_2CO_3 with a pK 6.1 (West and Todd, 1954) prevented a clear and accurate titration of the end point for lactic acid.

In a search of the literature for a desirable, primary standard, it was learned that Fossum et al. (1951) and Whitehead (1959) had all recommended tris (hydroxymethyl) aminomethane as an acidimetric standard. In examining the properties of this compound, it was found to have several advantages. The chemical is obtained in pure form (99.9%), is stable to mild heat, is non-hygroscopic and neither the aqueous or the solid form of this chemical will react with carbon dioxide. The equivalence point was reported to be at pH 4.7 when titrated with HCl. For standardizing strongly ionized inorganic acids, several indicators that change color at approximately this pH were recommended by Fossum et al. (1951) and Whitehead (1959). Potentiometric curves (Fig. 1) was carried out with hydrochloric and sulfuric acid standards, and confirmed the equivalence points to be at pH 4.6 for HCl and pH 4.7 for H_2SO_4.

Cucumber fermentations produce primarily lactic acid, but with a small amount of acetic acid. To establish the best end point for lactic and acetic acids and a 3:1 mixture of these two acids, potentiometric curves were determined for each. 5-ml amounts of 1.0 N tris (hydroxymethyl) aminomethane were titrated with 0.10 N concentrations of the acids; the plots are shown in Figure 2. The equivalence point for lactic acid is at pH 5.75, for acetic acid at 6.30 and for a 3:1 lactic-to-acetic mixture at 6.

Selection of indicator

Indicators recommended for the tris compound by Fossum et al. (1951) and by Whitehead (1959) were first considered, but they were ruled out because of their distinctive color change or end point at approximately pH 4.70 and below. In search of an indicator which would give a clear and distinct change at pH 5.8–6.0, both brom-thymol blue and phenol red were examined and found to be acceptable. Brom-thymol blue changed from dark-blue (alkali) to green, then to a distinct yellow at pH 6. Phenol red changed from cherry-red at pH 7.0 to orange, then to a very bright yellow at pH 5.8.

Test paper for 0.60% lactic acid

By calculation, 40.35 mg of tris (hydroxymethyl) aminomethane is equivalent to 5.0 ml of 0.60% (0.0667 N) lactic acid. In a series of tests with No. 1 Whatman paper, impregnated with different amounts of the tris compound and 0.05% phenol red, it was determined experimentally that a 25% (w/v) solution of the chemical gave an equivalence to the test acid when the dried paper was cut into 1.25- by 1.25-in. squares (32 by 32 mm). Fifteen test squares, representing the different sections of the 46- by 28.5-cm sheet of paper, were tested with the acid to determine variability of the coating of the chemical. Of the two methods used for impregnating the paper with the chemical, as described under Materials and Methods, the second proved to be the best, because it provided the least deviation in coating the chemical to the paper. Results on the use of the two methods for impregnating the chemical are shown in Table 1. The second method, using a 29.0% concentration of the chemical with 0.05% phenol red, gave the least variation in the impregnated paper and with an acceptable range of equivalent lactic acid (0.585–.612%) or ± 2% of 0.6%.

Commercial brine testing

Thirty-two cucumber brines, selected during the early part of the active fermentation period, were collected at three pickle companies. The quick-brine-acidity-test was determined at the time the samples were withdrawn from the vat;

Table 1—Variation of tris (hydroxymethyl) aminomethane in the test paper

Method	Tris (hydroxymethyl) aminomethane solution	Test paper squares cut from sheet at	Lactic acid equivalent for test paper[a] (avg or range)	Results with 0.60% lactic
	(% [w/v])		(%)	
1) Dipping, then air-drying vertically	25.0	Top	0.540	Low
	25.0	Bottom	0.660	High
	25.0	Middle	0.570–.630	Low and high
2) Chromatography tray and glass rod, then drying on freezer paper	25.0	All sections	0.510–.558	Low
	27.5	All sections	0.552–.588	Low
	29.0	All sections	0.585–.612	Acceptable
	32.5	All sections	0.655–.720	High

[a]Test papers were cut exactly into 1.25-in. squares. Average of three test papers or range for 15 test papers.

Table 2–Quick-brine-acidity-test as compared to titratable acidity of commercial cucumber brines

Pickle company	Brine samples collected	Brine color and acidity: Red Samples	Red Lactic Acid	Orange Samples	Orange Lactic Acid	Yellow Samples	Yellow Lactic Acid
	(No.)	(No.)	(%)	(No.)	(%)	(No.)	(%)
A	10	6	0.25–.52	1	0.59	3	0.64–.75
B	12	3	0.11–.39	1	0.52	8	0.62–.78
C	10	3	0.31–.42	1	0.59	6	0.66–.86

Table 3–Acidity of 255 commercial pickle-brine samples as measured by actual titration and by the quick-brine-acidity-test[a]

Total acid as lactic in brine by titration (%)	Brines tested[b] Red	Orange	Yellow
	(No.)		
0.45 and Less	75	0	0
0.50	9	8	0
0.55	4	7	3
0.60	0	12	7
0.65	0	6	11
0.70	0	3	17
0.75 and Above	0	0	93
Total	88	36	131

[a]Brine samples were tested by nine pickle companies located in Arkansas, California, Massachusetts, Michigan, Minnesota, North Carolina, Texas, Washington and Wisconsin.

[b]Red = negative = less than 0.5%; orange = about .50–.55%; and yellow = positive 0.6% lactic and higher.

and, also, the titratable acidity as percent lactic acid was made on preserved samples brought back to the laboratory. Results (Table 2) of these tests are in agreement, as shown by the range of titratable acidity under the specific color of the quick-test. The Red (negative) color corresponded to an acid range of 0.11–0.52% lactic; the Orange from 0.52–0.59% and the Yellow from 0.62–0.86%.

To further evaluate the commercial usefulness and accuracy of the test, certain pickle companies were given an experimental set of 40 Q-BAT papers along with a set of instructions. For a few companies, the test papers were folded and placed into disposable test tubes with rack to make the test operations simple to execute (KIM-RAK, 50 tubes, 16 by 100 mm with rack, all disposable after use and supplied by Kimble Products, Owens-Illinois, Toledo, OH 43601). The results are summarized in Table 3. In comparing acidity values determined by the titration method with those obtained by the brine color test using the Q-BAT papers, a good correlation was observed. Of the 255 samples, 88 contained 0.55% lactic acid or less and were Red, or negative, and 131 samples were Yellow, or positive, and three of these contained 0.55% acid, with the remaining 128 samples falling into the range of 0.6% and higher. Undoubtedly, there were some slight variations in technique between laboratories of the cooperating pickle companies. Also, there was possibly some variability with tests due to uneven application or migration during drying of the reagent. In the first samples of the Q-BAT papers, there was more deviation between individual papers as to the measurement of 0.6% lactic acid.

In general, over-all results for the new test are good. The person performing the test should realize that the Q-BAT method is only a quick test for brine acidity and not an exact quantitative acid measurement. The operator should use the test for each fermenting vat of cucumbers, and after obtaining a positive (Yellow color) for tests on 2 or 3 consecutive days, indicating 0.60% lactic and above, he can begin increasing the salt concentration of the brine. Comments by the pickle companies who cooperated in making these brine acid tests were generally most favorable. Additional test papers will be available to companies on a limited basis for further testing. We hope that a manufacturer will become interested in the preparation and supply of the Q-BAT papers. It may also be possible to use a quickly disintegrating tablet, pill or capsule containing known amounts of the test chemicals for making the brine test.

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