Sugar Fermentation Loss and Solubility, Enzyme and Color Losses Reported

BEET SUGAR LIQUORS

Determination and Concentration of Lactic Acid in Processing Liquors

J. B. STARK, A. E. GOODBAN, and H. S. OWENS

Western Regional Research Laboratory, Albany, Calif.

Lactic acid has been detected in processing liquors from factories located in all the major beet-producing areas. Ion exchange resins have been used to purify the solutions prior to colorimetric determination of lactic acid. The amounts of lactic acid in diffusion juices have varied from 30 to 600 mg. per 100 grams of sucrose. Whether this acid is an artifact or a natural constituent of sugar beets assumes importance when the possible dollar loss to the industry is considered. Examination of cossettes and factory diffusion juices prepared from them showed that the cossettes contained less than 6 mg. of lactic acid per 100 grams of sucrose, while the corresponding diffusion juices contained 10 to 100 times as much. Samples of liquors have been taken from various cells in continuous and batch diffusers to locate the site of possible fermentation.

LACTIC ACID IS ONE OF THE MAJOR NONSUGAR CONSTITUENTS present in the sugar-beet processing liquors that have been examined in this laboratory (*6*, *7*), but has been reported by Janacek to be absent from sugar beets (*3*). If it is found in processing liquors, that report assumes that it is the result of the action of lime on sucrose (*3*). The presence of lactic acid in thick juice or molasses could be attributed to this cause, but its presence in diffusion juice indicates that is is either a normal constituent of beets or is formed by fermentation. Representatives of the industry have generally assumed that fermentation is the source of the lactic acid, but its extent may not be fully realized (*4*, *8*).

If fermentation is the source of lactic acid, at least an equal amount of sugar, sucrose or invert, must have been utilized by the microorganisms concerned in producing lactic acid. The magnitude of the loss is apparent when the possible dollar loss is considered. If sucrose is involved as the substrate, the presence of 100 mg. of lactic acid per 100 grams of sugar will mean a direct loss of $250,000 annually to the industry. Because lactates are melassigenic and decrease the effectiveness of purification (*4*), at least as much additional sugar will go into molasses. In most of the diffusion juices examined at this laboratory the concentration of lactic acid has exceeded 0.1% on sugar (*6*, *7*). In view of this finding, a more detailed study of the problem of the extent and site of lactic fermentation was undertaken.

Collection and Preparation Of Samples

In 1951, cossettes were collected at four factories and preserved immediately in 5 volumes of boiling isopropyl alcohol. Later, the alcohol was removed by evaporation and the cossettes were extracted with water. Factory diffusion juice obtained from similar cossettes was concentrated to 70% solids. Preservatives, phenyl mercuric nitrate or a chlorophenol derivative, were added during collection of the samples and ahead of the concentration step to eliminate fermentation subsequent to diffusion, which might have been a factor in earlier results (*6*). The California samples were treated with toluene and transported directly to the laboratory without concentration. All samples were stored at 4° C.

During the 1952 campaign samples were obtained from 12 factories in order to obtain a broader survey of the problem. The site of fermentation in the diffusion batteries was also studied. Samples were withdrawn from various cells in four Robert batteries and two continuous diffusers and preserved immediately with toluene. Several weeks later a check was obtained on one of the Robert batteries by withdrawing and analyzing a second series of samples. A third set of samples was taken from the same battery after the temperature in the cells at the pulp end of the battery had been raised.

Determination of Lactic Acid

At present, pH measurement is widely used in the industry as an indication of fermentation, but there are several disadvantages. The buffering action of sugar beet juice will depend on its composition, so that a decrease of 0.2 pH unit may be more serious in juice from

one area than another. Figure 1 shows how much lactic acid is required to change the pH value of typical diffusion juices through a fairly narrow pH range. It is apparent that if a leeway of 0.5 unit is allowed in factory operations, loss of sugar will be significant. Because of the variation in the pH of the battery supply water and the composition of the beets, it is practically impossible to set a standard pH value at which a diffusion battery should operate; therefore the best control method would be direct measurement of lactic acid.

All lactic acid determinations in this laboratory have been carried out on samples purified by use of ion exchange resins. Samples from the 1951 campaign were analyzed after fractionation on an anion exchange resin in the cycle acid (*5*). This technique, while permitting the simultaneous determination of many acidic constituents, requires several days for completion. When it became apparent that the production of lactic acid was a serious problem, it was also apparent that a rapid method suitable for factory control work would be very useful. Such a method has been developed. In outline, it consists of removing amino acids on a cation exchange resin, sorbing all acids on an anion exchange resin, and washing out the neutral materials with water. An adaptation of the method used by Bryant and Overell (*2*) for the separation of succinic and citric acids has been found satisfactory for eluting the lactic acid, leaving the interfering materials on the resin. Lactic acid is determined by the colorimetric method of Barker and Summerson (*1*). Elution of the resin with formic or stronger acid, or with base, is not satisfactory, because formic acid interferes with the production of color, and the others elute interfering materials.

Materials and Reagents

Dowex-50 cation exchanger, 60- to 100-mesh, hydrogen form, cross linkage 12% (Dow Chemical Co.).
Amberlite IRA-400 anion exchanger, 60- to 100-mesh, carbonate form (Rohm & Haas Co.).
Ammonium carbonate, 0.25 *N* (14.25 grams per liter, lump ammonium carbonate).
Sulfuric acid, concentrated reagent grade, nitrate-free.
p-Hydroxydiphenyl, 1% in 0.1 *N* sodium hydroxide.
Standard lactate solution.
Sodium carbonate, 1 *N*.
Copper sulfate, 4%.

Procedure

Columns used are borosilicate glass 14 mm. in outside diameter, stoppered at one end with a one-hole No. 00 rubber stopper and a short piece of glass tubing. A small circle of nylon bolting cloth

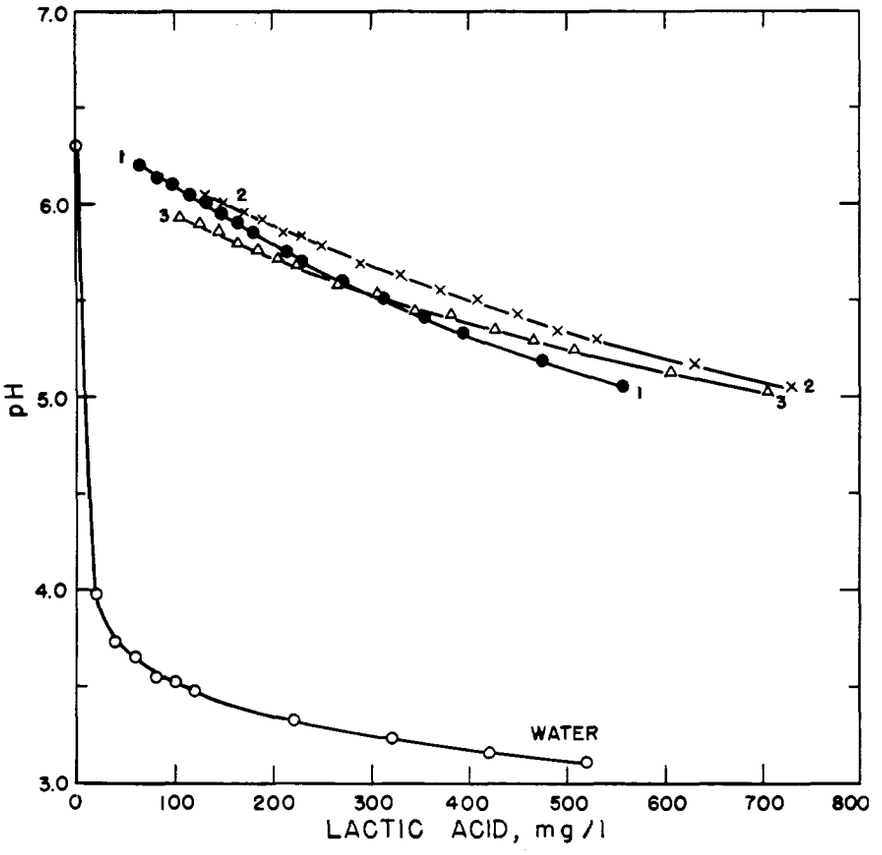

Figure 1. Buffering capacity of typical diffusion juices for lactic acid

1. Minnesota, initial lactic, 66 mg. per liter
2. Colorado, initial lactic, 130 mg. per liter
3. California, initial lactic, 105 mg. per liter

serves to retain the resin in the column. No threads of the cloth should project below the bottom of the column. No stopcock or pinch clamp is necessary, as the column will not drain dry if the resin used is ground to 60- to 100-mesh. One column, 35 cm. long, is loaded with 10 ml. of IRA-400 carbonate; a second column, 20 cm. long, is loaded with 10 ml. of D-50 hydrogen form, supported above the first column so that the effluent drips directly from the cation column onto the anion column. An aliquot of the sample to be analyzed is added carefully to the upper column and allowed to run freely through both columns. The upper column is washed carefully with three 10-ml. portions of water. Each portion of wash water is allowed to drain through the column before the next is added. After washing, the upper column is removed and the lower column is washed with three 10-ml. portions of water. The lactic acid is now eluted from the IRA-400 with 75 ml. of ammonium carbonate solution. This may be done with 10-, 15-, 25-, and 25-ml. aliquots. The eluate is collected in a 100-ml. volumetric flask and made to the mark. An aliquot is heated with concentrated sulfuric acid and the resulting acetaldehyde determined with *p*-hydroxydiphenyl, according to the method of Barker and Summerson (*1*). The sample of beet liquor should contain not more than 3 meq. of total acid and not less than 0.2 mg. of lactic acid. For most cases 10 ml. of diffusion juice and 3 ml. of molasses at 10% sugar are adequate. For cell juices containing less total acid than diffusion juice, increasing amounts may be used.

Results and Discussion

The data in Table I show that cossettes contain very little lactic acid, while the corresponding diffusion juice contains 10 to 100 times as much. This proves that lactic acid is produced during diffusion. The extent of fermentation in diffusion

Table I. Lactic Acid Content of Diffusion Juice and Juice from Comparable Cossettes

(Mg./liter at 10% sucrose, 1951 campaign)

Factory	Sample	Location	Lactic Acid
2	D[a]	Colo.	125
2	C		5
3	D	Mich.	310
3	C		6
5	D	Calif.	412
5	C		3
6	D	Minn.	66
6	C		6

[a] D refers to diffusion juice and C to extract from cossettes.

Table II. Lactic Acid Content of Diffusion Juices

(Mg./liter at 10% sucrose, 1952 campaign)

Factory	Location	Lactic Acid
1	Calif.	145
2	Calif.	290
3	Colo.	100
4	Mich.	140
5	Utah	110
6	Calif.	160
7	Colo.	130
8	Calif.	240
9	Idaho	210
10	Mont.	600
11	Wash.	32
12	Calif.	250

juice from factories located in the major beet-producing areas is further shown in Table II.

In order to attack the problem more rationally it seemed advisable to know the site of the fermentation in the diffusion battery. The analyses of samples taken from a representative series of cells in three Robert batteries of 14 cells each and two continuous diffusers are given in Tables III and IV. The continuous diffuser at factory 4 had 21 cells and the diffuser at factory 5 had 24 cells.

Table III. Analyses of Samples in Robert Batteries

	Factory			
Cell No.	1, Sample A	1, Sample C	2	3
	Lactic acid, mg./liter of juice			
1	335	85	175	90
3	315	10	265	120
5	315	10	290	130
7	315	10	..	95
9	270	10	250	80
11	110	..	60	..

Table IV. Analyses of Samples in Continuous Diffuser

	Factory	
Cell No.	4	5
	Lactic acid, mg./liter of juice	
1	400	250
4	195	..
5	..	277
8	175	280
12	95	350
16	70	345
20	..	140

Samples A and C in Table III are from the same factory. A represents the lactic acid production with low temperature battery supply water. The battery supply was at 53° C., only the head cells being heated. Juice temperature did not reach 70° until the fifth cell, with a maximum of 85–87° in cell 2. A second set of samples from this factory (not included in the table) showed essentially the same levels of lactic acid. C shows the effect of increasing the temperature of the battery supply water to 62° and heating the cells at the tail end of the battery to obtain a temperature of 67° in the last cell and higher than 70° in all other cells. Under these conditions the juice taken from cell 3 and the others going toward the pulp end of the battery contained only 10 mg. of lactic acid per liter as compared to the earlier figure of 315 mg. per liter. The diffusion juice, however, contained 85 mg. per liter compared to 335 mg. per liter found earlier. In all cases the juice is considerably cooled when it comes in contact with fresh beets in the head cell. Considerable fermentation must take place in this cell in all batteries. In the case of C probably all the fermentation takes place in the first cell with concomitant diffusion into cossettes. In subsequent cells, the lactic acid is extracted from the cossettes. It appears that fermentation is more severe than is shown by the amount of lactic acid present in the diffusion juice, since some acid may be retained in the pulp.

The temperatures of the samples from factory 3 were also higher than for factory 1, sample A. The temperature for cell 9 was 69° C. with a maximum of 85° C. in cells 3 to 5. The lactic acid present in the diffusion juice was about the same as in factory 1, sample C, but it appears that a larger amount was carried out in the pulp.

The two factories with continuous diffusers appear to have two different fermentation sites. Much of the fermentation at factory 4 (21 cells) takes place at the beet end, while at factory 5 (24 cells) the highest lactic acid concentration is found in the center of the battery.

The data in Tables III and IV represent only momentary conditions in each cell in a continuous process. Although the batteries may be nearly in equilibrium with respect to sugar extraction, this is not necessarily true of lactic acid production. Lactic acid production may take place in varying amounts at several places in the system, while sugar is introduced only in the cossettes. The cossettes, the juice, or the cell itself may be heavily or lightly contaminated with bacteria-producing lactic acid. The juice and cossettes leaving this cell will have an effect on the cossettes and juices from the other cells. This effect will appear to some degree for a number of cycles, since some lactic acid is being carried forward with the juice while some diffuses into the cossettes to be extracted by the succeeding juice. For these reasons it is very difficult to locate precisely the sites of fermentation in various batteries without information as to the previous levels of lactic acid in the juice and pulp. The data presented, however, show that conditions for lactic fermentation must be favorable in the first cell under present operating procedures.

From the marked decrease in lactic acid production when temperatures of 70° C. or above are used in the diffusion battery, it is apparent that heat sterilization is one method of decreasing lactic fermentation. This method may offer disadvantages in factories operating pulp presses, particularly if the alkalinity of the battery supply water is sufficiently high to solubilize the polysaccharides in the beet cell walls. This would adversely influence operations throughout the factory, besides decreasing the pressability of the pulp (*9*).

Antiseptics, particularly formaldehyde, have been widely used with continuous diffusers but offer an economic disadvantage that tends to offset the gain in sucrose yield. However, increased diffusion temperature, addition of antiseptics, or a combination of these methods applied to the batteries should offer improved control of lactic fermentation. In order to eliminate fermentation in the head cell, these may be supplemented by following the occasional European practice of scalding the cossettes (*9*).

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