# Lactic Acid Fermentation in Beet Processing Juices

J. B. STARK, A. E. GOODBAN, AND H. S. OWENS[1]

Our first report (1)[2] to this group, in 1950, demonstrated that lactic acid is one of the major acidic constituents of some diffusion juices. A general composition study of samples from nine factories presented at the 1952 meeting of this Society (2), confirmed the presence of varying amounts of lactic acid. If the lactic acid is produced by fermentation, at least an equal amount of sugar must have been utilized by microorganisms. Because lactates are melassigenic and decrease the efficiency of crystallization (3) approximately one and one-half times as much additional sugar will be lost in the molasses. The formation of 100 mg. of lactic acid for every 100 g. of sugar (or 0.1 percent on sucrose) will mean a direct loss of $250,000 annually to the industry. Depending on the price of molasses, there will be an equal or even greater loss through increased molasses formation. The lactic acid concentration exceeded this value of 0.1 percent on sugar (1, 2) for most of the juices previously examined at this Laboratory.

## Materials and Methods

The general method of sample collection and preservation has been described in a previous publication (4). The methods of analysis are also described therein (4).

## Results and Discussion

The results of analyses of diffusion juice and juice from comparable cossettes show that most of the lactic acid present in diffusion juice is formed during the diffusion process (4).

Table 1.—Lactic Acid Content of Processing Liquors, 1952 Campaign (mg./liter at 10% Sucrose).

| Factory | Location | Diffusion juice | Molasses |
|---|---|---|---|
| 1 | Alvarado, Calif. | 145 | 1620 |
| 2 | Betteravia, Calif. | 290 | 3100 |
| 3 | Brighton, Colo. | 100 | 3000 |
| 4 | Carrollton, Mich. | 140 | 2490 |
| 5 | Centerfield, Utah | 110 | 2600 |
| 6 | Clarksburg, Calif. | 160 | 2970 |
| 7 | Loveland, Colo. | 130 | 3940 |
| 8 | Manteca, Calif. | 240 | 2750 |
| 9 | Moorhead, Minn. | 1185 | 5320 |
| 10 | Rupert, Idaho | 210 | 1480 |
| 11 | Sidney, Mont. | 600 | 3200 |
| 12 | Toppenish, Wash. | 30 | 1290 |
| 13 | Woodland, Calif. | 250 | 1740 |

Analyses of samples from the 1952 campaign (Table 1) showed contents of lactic acid in the diffusion juices similar to those observed in the 1951 campaign. It is apparent that the concentration of lactic acid is considerably greater in the molasses than would be expected from the amount in the diffusion juice. With the exception of the samples from factories 9

[1] Western Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture, Albany 6, California.

[2] Numbers in parentheses refer to literature cited.

and 11, there is roughly a 10- to 40-fold increase of lactic acid in molasses. Part of the unexpected increase in the molasses may be due to fermentation in holding tanks before heating and liming. Fermentation losses during holding can probably be decreased by heating the juice to above 70° C. as quickly as possible after leaving the diffusers and by cleanliness in the holding tanks and other equipment.

Part of this increase in lactic acid is due to chemical decomposition of sucrose by lime. Lactic acid is one of the principal decomposition products (5) of sucrose in highly alkaline solution. Honig (6) referring to work by Spengler and Todt (7) states, "In a solution of sucrose with lime of pH 12, the sugar loss in 1 hour boiling under normal pressure was found to be about 0.5 percent." The sugar loss is about three times higher per one unit rise in pH. The minimal decomposition of sucrose (6) takes place at approximately pH 9. Losses at this pH are about 0.05 percent for 1 hour boiling at normal pressure. A decrease of 10° C. in boiling temperature reduces this loss to about 0.02 percent or less.

On the basis of a six-fold increase of impurities from diffusion juice to molasses, it appears that approximately 200 mg. of lactic acid per 100 g. of sugar in diffusion juice is formed during liming and subsequent treatments at high pH. This shows the importance of not exceeding pH 9 or maintaining a high temperature for a longer time than is necessary.

It was desirable to know the site of fermentation during the diffusion process in order to evaluate the application of preventive measures. Tables 2 and 3 present the lactic acid analyses of cell juices from Robert batteries and continuous diffusers, respectively.

Table 2.—Lactic Acid in Samples from Robert Batteries (mg/liter of Juice).

| | Factory | | | |
|---|---|---|---|---|
| Cell No. | 8 Sample A | 8 Sample C | 1 | 6 |
| 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 3.—Lactic Acid in Samples from Continuous Diffusers (mg./liter of Juice).

| | Factory | |
|---|---|---|
| Cell No. | 2 | 13 |
| 1 | 400 | 250 |
| 4 | 195 | . |
| 5 | | 277 |
| 8 | 175 | 280 |
| 12 | 95 | 350 |
| 16 | 70 | 345 |
| 20 | ...... | 140 |

Samples A and C in Table 2 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 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 temperature of the samples from factory 6 were also higher than for factory 8, 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 8, 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 2 (21 cells) takes place at the beet end, while at factory 13 (24 cells) the highest lactic acid concentration is found in the center of the battery.

The data in Tables 2 and 3 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 contaminated with bacteria capable of 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 pasteurization 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 (8). Recent attempts to control non-yeast fermentations in breweries and alcohol fermentations have been fairly successful (9, 10). Strandskov and Bockelmann (10) report the control of gram-negative rods by polymyxin in concentrations of 0.005 mg./liter. If this antibiotic could be successfully applied in diffusion at this concentration it would be less expensive than formaldehyde.

The finding of so much lactic acid in diffusion juice raises the question of other fermentations. In at least two of the processing liquors, significant amounts of dextrans were found (2). Freed and Hibbert (11) report the formation by enzymatic synthesis of oligosaccharides and polysaccharide gums by the bacteria, yeasts, and fungi present on or in frosted beets. No doubt other products of different organisms could be found on closer examination so that a real problem for the beet industry exists and warrants further study.

Table 4.—Lactic Acid in Samples from Factory No. 8 at Different Collecton Times (mg./liter at 10% Sucrose).

| Sample | Date of sampling | Diffusion juice | Molasses |
|---|---|---|---|
| A | 9/7/52 | 240 | 2750 |
| B | 10/7/52 | 190 | 2290 |
| C | 11/17/52 | 70 | 1360 |

Table 4 presents the analyses for 3 diffusion juice and molasses samples that were obtained from the same factory at intervals o,£ two or three weeks. Samples A and C are the same as those given in Table 2. When the amount of lactic acid is decreased in the diffusion juice there is a corresponding decrease in the molasses. There is approximately 1 to 1 1/2 grams of lactic acid per 100 g. of sugar in molasses that is formed by other means than fermentation in the diffusion battery.

Table 5.—Comparison of Lactic Acid in Processing Juices (mg./liter at 10% Sucrose).

| Factory | Diffusion juice | Thick juice | Molasses |
|---|---|---|---|
| 1 | 145 | 220 | 1620 |
| 2 | 290 | 750 | 3100 |
| 8 | 190 | 430 | 2290 |

Table 5 compares the quantity of lactic acid in diffusion juice, thick juice, and molasses. It is evident that most of the increase in lactic acid after diffusion takes place before or during concentration of the liquor to thick juice.

**Summary** A comparison of lactic acid concentrations has been made of cossettes, diffusion juice, thick juice, and molasses. Lactic acid is formed during diffusion, probably by fermentation, which may occur throughout the battery.

A considerable portion of the acid is formed in the head cell. Increasing the temperature of the battery supply water markedly decreases the lactic fermentation. The high concentrations of lactic acid in molasses indicate alkaline degradation of sucrose during defecation and carbonation.

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