Fermentation Method for Production of Dextro-Lactic Acid

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IN SPITE of the present large-scale production of edible lactic acid by fermentation processes, dextro-lactic acid, the form found in animal tissues, is both limited in supply and almost prohibitively expensive. Dextrorotatory lactic acid, because of its function in animal metabolism, is the form in which physiologists and biochemists are primarily interested, and if it could be made readily available, would be of great value scientifically. What *d*-lactic acid is available, however, is made by chemical resolution of commercial lactic acid, which is optically inactive—that is, it is a mixture of the dextro- and levorotatory acids.

A more important use of *d*-lactic acid in large quantities would be as a foodstuff, since the dextro isomer is metabolized completely by the animal body, while the levo form is largely excreted as such (*1*). This means that when the so-called edible lactic acid (inactive acid) is used as a food or as an addition to food, the dextro component is used by the body, while the levo acid is largely unavailable. Therefore, the commercial inactive acid is only about one-half edible in the true sense of the word. Consequently, if *d*-lactic acid were as available as ordinary lactic acid, it would undoubtedly have a large market as a food.

For the past 5 or 6 years *d*-lactic acid has been made in this laboratory by fermentation in pound lots, approximately 20 pounds (9.1 kg.) in all, and has been furnished to a number of investigators for experimental purposes. From their experience the authors feel that commercial production could be carried out easily and successfully, without necessitating any very great change in present plant equipment or procedure. The ordinary commercial sirupy lactic acid is produced from sugar by fermentation with lactic acid bacteria of the *L. delbruckii* type. The fermentations are carried out in open vats, the only control of contaminating organisms being the high acidity which is developed rapidly in the medium, and the relatively high temperature of incubation (37° to 45° C.). The product is a mixture of the two forms of lactic acid, dextro and levo, because the organisms used produce both forms and any contaminants present either produce both forms of acid or may cause the lactic organism to produce both types (*6*).

The type of lactic acid formed by an organism seems to be characteristic of that organism. Thus organisms have been isolated which in pure culture produce *l*-lactic acid, some which form dextro acid, and a majority which give inactive acid (*5*). The organism or mixtures of organisms used in the commercial production of lactic acid form inactive acid (*4*), a mixture of both types. There are in the authors' laboratory strains of lactic acid-producing organisms some of which form levo acid, some dextro acid, and others mixtures of both forms (*6*).

The production of *d*-lactic acid on a commercial scale by fermentation would therefore necessitate only the selection of the proper strain of organism for fermentation. If the right strain is used and contaminating strains rigorously excluded by adequate bacteriological control of the process, there should be no difficulty in producing *d*-lactic acid on a large scale. In order to insure this rigid bacteriological control the medium would have to be sterilized and fermented in closed vats which could be kept sterile. Large-scale fermentations of this sort have been carried out for many years in various industries, notably in the production of butyl alcohol and acetone from corn (*3*).

In the production of *d*-lactic acid in this laboratory, the authors have used a medium containing 3 per cent cerelose and 3 per cent malt sprouts as a source of nitrogen. The fermentations were carried out in 18-liter batches in 20-liter Pyrex bottles. After sterilization the medium was inoculated with a *d*-lactic acid-producing culture (in most cases a strain of *L. delbruckii*) and incubated at 30° or 37° C., depending on the organism used. After 24 hours an excess of sterile calcium carbonate was added and the fermentation allowed to proceed for 6 to 10 days with frequent shaking to neutralize the acid formed. At the end of the incubation practically all the sugar was fermented, and the yield of acid was from 90 to 95 per cent of the sugar used. After acidification and filtration, the lactic acid was extracted with ether in a continuous extractor, converted into the zinc salt, and a sample analyzed for its specific rotation and its water of crystallization. The data for some of these fermentations are given in Table I. In all cases the acid formed was pure dextro acid.

TABLE I. LACTIC ACID PRODUCTION BY VARIOUS ORGANISMS

Organism	Temperature of Incubation of Glucose	Lactic Acid Formed per 540 Grams of Glucose	Glucose Converted to Lactic Acid	Analysis of Zinc Lactate: Water of crystallization	Analysis of Zinc Lactate: Specific rotation [a]	Form of Lactic Acid
	° C.	*Grams*	%	%	$[\alpha]_D^{20}$	
S. lactis, R	30	495	91	12.52	−8.65	Dextro
S. lactis, R	30	525	97	13.23	−8.65	Dextro
L. casei	30	505	93	12.80	−8.22	Dextro
L. delbruckii, 3	37	530	98	13.00	−8.22	Dextro
L. delbruckii, 3	37	520	96	12.90	−8.18	Dextro
L. delbruckii, 3	37	500	92	12.86	−8.60	Dextro

[a] 4 per cent concentration.

TABLE II. FERMENTATION OF GLUCOSE, MALT SPROUTS MEDIUM BY *L. delbruckii*, 3

Medium: Glucose	Medium: Malt Sprouts	Glucose Fermented	Lactic Acid[a]	Lactic Acid Formed per 100 Grams Glucose Fermented
G./100 cc.	*G./100 cc.*	*G./100 cc.*	*G./100 cc.*	*Grams*
3	3	2.95	2.74	92.8
5	3	4.93	4.56	92.7
10	3	9.54	8.72	91.8
3	5	2.91	2.93	100.5
5	5	4.90	4.69	95.8
10	5	9.87	9.07	92.0

[a] Lactic acid determined according to Friedemann and Graeser (*2*).

In producing *d*-lactic acid on a commercial scale the concentration of sugar could be increased considerably, certainly up to 6 or 8 per cent and possibly to 10 per cent. The results

of a few experiments using varying sugar and malt sprout concentrations are shown in Table II. Over 90 per cent of the sugar was recovered as lactic acid when 3, 5, or 10 per cent of sugar was used. All the fermentations were completed in 8 days. Since these fermentations were conducted at 37°, a higher temperature—e. g., 45° C.—would undoubtedly result in a more rapid fermentation. The higher concentration of malt sprouts increased the yield of acid slightly, probably because of the fermentable material present in the malt sprouts which was converted to lactic acid. After fermentation the medium could be worked up as is now done commercially, by acidification with sulfuric acid, filtration, and vacuum concentration. Such treatments will not affect the form of lactic acid.

The raw materials used by the authors (cerelose and malt sprouts) are relatively inexpensive, so that the cost of producing the active acid should be low. Moreover, any of the materials (malted grain, molasses, etc.) from which lactic acid is now made commercially undoubtedly could be used equally well for the production of dextrorotatory lactic acid.

The organism producing the dextro form is just as vigorous and efficient as organisms producing the inactive form. Except for care in the exclusion of foreign organisms, the commercial production of *d*-lactic acid should be no more difficult than that of the inactive form.

Literature Cited

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RECEIVED July 13, 1935.

Changes in Stored Corn Meal

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Corn meal may be stored for a long period of time without change in the crude fat content: (*a*) When the moisture content is 14 per cent or higher, the storage temperature must be maintained at 18° C. or lower; (*b*) with a moisture content less than 8 per cent, the storage temperature may be as high as 37° C.

The "degrees of acidity" of corn meal do not indicate changes in crude fat and therefore may not be used as an index of crude fat losses.

IN THE work of the New York State Feed Control Laboratories, it was noted that some feeds had apparently undergone a loss in crude fat content during the interval between the manufacturer's analysis and the check analysis made at the laboratories. These apparent losses ranged from a few tenths to more than 1 per cent. Information concerning the cause of these conditions would not only be helpful to the feed manufacturer and dealer, but also to the various control laboratories. The manufacturer would be able to guarantee the crude fat content with a margin of safety, and the dealer and control laboratory would be able to store feed or feed samples under conditions which would cause only a minimum of change.

The purpose of this investigation was to determine changes in fat and acidity in white corn meal during storage, believing that such a study might indicate changes in other fat-containing animal feeds. Corn meal was chosen since it is more or less uniform, and because an adequate supply was available.

Previous Investigations

The changes in stored whole corn and corn meal have been studied by several investigators. But since these changes were observed in large commerical quantities, the temperature conditions of storage were not controlled; they were either the seasonal changes in temperature which the corn in the storage bins underwent, or the temperature changes to which it was subjected during shipping. Studies of acid production in stored corn meal have been made but at relatively few storage temperatures (*5*). No work has been done on changes in the crude fat content which occurs in corn meal during long periods of storage.

In most of the previous work, emphasis was placed upon a correlation of the deterioration of corn with its gain in acidity. Black and Alsberg (*4*) stated in 1910 that the degree of acidity is an index of the work of microörganisms and that the samples of highest acid content also had the highest content of fat and nitrogen. This was due to a loss in some constituent, supposedly carbohydrate, to which they attributed the gained acidity.

Duvel (*7*) in 1909 compared the relative keeping qualities of dried shelled corn with the undried product and found that the dried corn kept 14 days longer without spoilage.

The keeping qualities of ton lots of ground stored corn meal were studied in 1915 by Winton, Burnet, and Bornmann (*11*). They found that corn meal of less than 15 per cent moisture underwent almost no change in taste or appearance. This work was checked by Davies (*6*) in 1928.

McHargue (*8*) found in 1920 that in the presence of air and excessive moisture, deterioration in the qualities of corn occurred at low temperatures and that corn meal, deprived of its moisture, underwent little or no change in acidity.

A continuation of the studies, begun by Winton and others, was made by Bailey and Thom (*2*) in 1920. Samples of corn meal were stored for 5 months in a cool room. All the samples underwent a gradual decrease in moisture, while the acidity increased more or less regularly. Samples stored in the laboratory for the same period of time showed that those with a higher moisture content had a corresponding increase in acidity and mold development.

Storage Conditions

The following investigations were undertaken to determine some of the effects of moisture and temperature on the crude fat (ether extract) and acidity of stored corn meal: