inert atmosphere. Further advantage may result from the addition of antioxidant during fermentation.

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OXALATE IN BEER

The Rapid Enzymatic Determination of Oxalate in Wort and Beer

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A rapid manometric procedure for determination of oxalate in wort and beer employing the enzyme oxalic decarboxylase is described whereby oxalic acid is quantitatively decarboxylated to formic acid and carbon dioxide. Fluoride and cyanide were required as inhibitors in the presence of carbohydrate. Levels of fluoride and cyanide are critical. Excess of either alters the pH, causing low or negative results. Recovery studies were performed and average recovery was found to be 100.9% in wort, 100.2% in beer, 102% in deionized beer, and 99.8% in water. Good correlation was found between the enzymatic procedure and the precipitation-titration method.

A RAPID and accurate method for quantitative determination of oxalic acid in beer and wort has long been required. Burger and Becker (*4*) reported that sediment formed in beer on prolonged storage was in part calcium oxalate. Brenner (*3*) stated that this haze- and sediment-forming material may be a cause of gushing. Burger and Becker (*5*) mentioned oxalate as one cause for nonbiological type haze formed in old beer. In 1956, Burger, Glenister, and Becker (*6*) showed definitely that the oxalate to calcium ratio in beer is a measure of the tendency of that beer to gush.

Usual methods for quantitative determination of oxalates involve their precipitation as calcium salt. This salt may be converted into the oxide and weighed or titrated with standard acid (*4*). Yarbro and Simpson (*9*) titrated precipitated calcium oxalate with standard potassium permanganate. Permanganate titration gives an indistinct end point and high results due to interaction of permanganate with occluded organic materials in the precipitate (*6*). A 40-hour precipitation period in the cold is required (*6*) and a correction factor for the solubility of calcium oxalate must be applied (*1, 2*).

Shimazono and Hayaishi (*7*) first suggested use of oxalic decarboxylase as an analytical tool. It was used by us in developing the method described herewith, which employs the enzyme oxalic decarboxylase. Oxalic acid is quantitatively converted by this enzyme to formic acid and CO_2 according to the reaction:

$$HOOC—COOH \rightarrow HCOOH + CO_2$$

The CO_2 evolved was measured manometrically.

Experimental

Reagents. Potassium citrate buffer, 0.2*M*, pH 3.2.

Oxalic decarboxylase, prepared according to the procedure of Shimazono and Hayaishi (*7*).

Oxalic acid, 10 μmoles per ml.

Sodium fluoride, saturated aqueous solution.

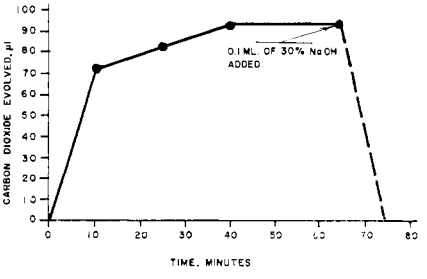

Figure 1. Rate of oxalic acid decomposition by 100 units of oxalic decarboxylase

Potassium cyanide, 10% aqueous solution.

Equipment. Warburg respirometer with single arm vessels (either plugged or vented).

Procedure. In the main compartment of a Warburg vessel are placed 3 ml. of sample (beer is first degassed), 0.1 ml. of sodium fluoride, 0.1 ml. of potassium cyanide, and 0.6 ml. of citrate buffer. One hundred units of oxalic decarboxylase dissolved in 0.2 ml. of

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Table I. Recovery of Oxalic Acid from Water, Deionized Beer, Beer, and Wort

Sample	Oxalic Acid, μMoles Added	Oxalic Acid, μMoles Recovered	Recovery, %
Water	0.50	0.50	100
	0.90	0.93	103
	0.98	0.99	101
	1.50	1.35	90.3
	1.50	1.51	100.8
	1.90	1.80	95.0
	1.96	1.97	100.5
	2.94	2.98	101
	3.00	2.92	97.4
Deionized beer	0.00	0.00	...
	0.50	0.505	101
	1.00	1.04	104
	1.50	1.58	105
	1.80	1.74	97
	2.00	2.09	104
	2.50	2.60	104
	3.00	3.08	102
Wort[a]	0	0[b]	...
	1.0	1.04	104
	2.0	1.98	99.2
	3.0	2.99	99.8
	4.0	3.99	99.9
	1.0	1.03	103
Beer[c]	1.00	1.01	101
	2.00	2.01	100.5
	2.10	2.10	100
	2.90	2.87	99
	3.00	3.01	100.3

[a] Wort initially contained 0.64 μmole of oxalic acid per ml.
[b] All values below corrected for sample oxalic acid concentration.
[c] Beer initially contained 0.21 μmole of oxalic acid per ml.

Table II. Comparative Determination of Oxalic Acid in Wort by Enzymatic and Precipitation-Titration Methods

Sample	Oxalic Acid, P.P.M. Enzymatic method	Oxalic Acid, P.P.M. Precipitation-titration method
A	32.6	32.6
B	21.8	20.7
C	21.2	22.0

water are added to the side arm. A unit of oxalic decarboxylase is defined as that quantity of enzyme which will decompose 1 μmole of oxalic acid in 1 hour at 37° C. The vessel, attached to the manometer, is placed in the bath and permitted to equilibrate at 37° C. for 15 minutes. The manometer is closed and the system permitted to attemperate for an additional 5 minutes before a zero time reading is taken and the enzyme is tipped into the cell. The cells are agitated at 100 shake cycles per minute. Readings are taken at 15-minute intervals until evolution of carbon dioxide ceases, usually after 1 hour (Figure 1). A thermobarometer and a standard oxalic acid solution must be run simultaneously with each series.

Calculation.

$$\frac{(A_2 - A_1)K_{CO_2}}{22.4} = \text{micromoles of oxalic acid in the sample}$$

where
A_1 = manometer reading at time zero
A_2 = final manometer reading corrected for the thermobarometer
K_{CO_2} = cell constant (*8*)

Results and Discussion

Figure 1 shows that only carbon dioxide is evolved in the reaction, by adding 30% of sodium hydroxide to the system through the vent tube which quantitatively reabsorbs carbon dioxide.

Recovery studies on oxalic acid in water are shown in Table I.

Beer was deionized by passing it through a strong mixed bed of ion exchange resin, a Deminac deionizer (Crystal Research Laboratories, Inc.). The effluent was adjusted to pH 3.0. Known amounts of oxalic acid were dissolved in this oxalate-free beer which were then analyzed for oxalic acid content. Average recovery was 102%.

Recovery studies were run on wort and beer (Table I). Average recovery was 100.9% in wort and 100.2% in beer. Oxalic acid was determined in some worts using both the enzymatic and the precipitation-titration methods of Burger and Becker (*4*). Results are given in Table II and show good correlation.

When sodium fluoride and potassium cyanide were omitted from the system, abnormally high values for oxalic acid were obtained. In a system without oxalic acid, but containing only maltose, 0.2*M* citrate buffer, and enzyme, 63 μl. of CO_2 were liberated and 38.4 μl. of O_2 were taken up in 180 minutes. Addition of 0.1 ml. of a saturated solution of sodium fluoride (which acts by precipitating magnesium, a cofactor needed for enzymatic oxidation of carbohydrates to CO_2) completely inhibited formation of CO_2 from carbohydrates, without inhibiting quantitative decarboxylation of oxalic acid. Addition of 0.1 ml. of a 10% sodium cyanide solution completely inhibited oxygen uptake without inhibiting quantitative decarboxylation of oxalic acid.

The amount of inhibitors in the vessel is critical. Required amounts ranged from 0.05 to 0.1 ml. for both fluoride and cyanide per Warburg cell with a final total volume of 4 ml. When fluoride and cyanide were supplied in excess, inaccurately low and even negative results were obtained. In both cases, this result was due to absorption of CO_2 caused by an increase in pH (which results from addition of these inhibitors beyond the buffering capacity of the system). This pH effect is considerably more pronounced with cyanide ion than with fluoride ion.

In the absence of carbohydrate, neither cyanide nor fluoride was required.

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