

## LACTIC ACID

### PRINCIPLE

Lactic acid in steepwater (Note 1) is oxidized to acetaldehyde following treatment with copper sulfate and calcium hydroxide to remove interfering substances. Acetaldehyde is distilled and absorbed in aqueous sodium bisulfite solution; the stoichiometrically combined bisulfite is determined iodometrically.

### SCOPE

The method is applicable to the determination of lactic acid and lactate salts (Note 2) in light or heavy steepwater.

### SPECIAL APPARATUS

Distillation Apparatus: This consists of a 300 mL boiling flask to which a dropping funnel (50 mL capacity) and a connecting bulb (Kjeldahl type) are attached by means of a rubber stopper. The arm of the connecting bulb is attached to a vertical, water-cooled condenser by means of rubber tubing. The condenser delivery tube is sufficiently long to extend to the bottom of a 125 mL Erlenmeyer receiving flask. The boiling flask is heated by an electric heater or a microburner (see sketch).

### REAGENTS

1. Copper Sulfate Solution, 20%: Dissolve 200 g of reagent grade copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in purified water and dilute to 1 L volume.
2. Calcium Hydroxide Suspension: Cautiously slake 200 g of reagent grade calcium oxide (CaO) with a small amount of purified water and immediately dilute to 1 L volume. Shake vigorously prior to use, allow to settle a few seconds and use an aliquot of the suspension which is free of coarse particles.

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3. Manganous Sulfate-Phosphoric Acid Reagent: Dissolve 100 g of reagent grade manganous sulfate tetrahydrate ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ) in 500 mL of warm purified water and add 25 mL of concentrated reagent grade phosphoric acid (85%  $\text{H}_3\text{PO}_4$ , sp g 1.7). Cool and dilute to 1 L volume with purified water.
4. Sodium Bisulfite Solution, 1.25%: Dissolve 12.5 g of reagent grade sodium bisulfite ( $\text{NaHSO}_3$ ) in purified water and dilute to 1 L volume. Store in a glass stoppered bottle. Prepare fresh weekly.
5. Potassium Permanganate Solution, 0.01 N: Dissolve 0.320 g of reagent grade potassium permanganate ( $\text{KMnO}_4$ ) in purified water and dilute to 1 L volume. Store in amber glass bottle. Prepare fresh weekly.
6. Iodine Solution, 4%: Dissolve 60 g of reagent grade potassium iodide (KI) in about 45 mL of purified water, add 40 g of iodine crystals and stir until the solution is complete; dilute to 1 L volume. Store in amber glass-stoppered bottle.
7. Iodine Solution, 0.01 N: Standard
8. Sodium Thiosulfate Solution, 0.1 N: Dissolve 25 g of reagent grade sodium thiosulfate pentahydrate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in purified water, add 0.5 g of sodium carbonate and dilute to 1 L volume.
9. Sodium Bicarbonate Solution, Saturated: Add 115 g of reagent grade sodium bicarbonate ( $\text{NaHCO}_3$ ) to 1 L of purified water at room temperature; stir mechanically for 30 mins. and allow the undissolved crystals to settle. Use only the clear supernatant solution.
10. Sodium Carbonate Solution, 10%: Dissolve 100 g of reagent grade anhydrous sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) in purified water and dilute to 1 L volume.
11. Starch Indicator Solution: Lintner 1% in purified water.

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Weigh accurately about 2 g of commercial steepwater (Note 3), dilute to volume with purified water in a 200 mL volumetric flask, and mix thoroughly. Pipet 25.0 mL of this solution into a 200 mL centrifuge bottle, add 8.0 mL of copper sulfate solution, 8.0 mL of calcium hydroxide suspension and 159 mL of purified water (total volume 200 mL). Close bottle with a rubber stopper, shake mixture for 1 min. and centrifuge immediately for 5 mins. at 2000 rpm.

Pipet 20.0 mL of supernatant solution (aliquot should contain not more than 5 mg of lactic acid) into boiling flask. Add 10 mL of manganous sulfate-phosphoric acid reagent and sufficient purified water to bring the total volume to 100 mL. Add a pinch of talcum to prevent bumping during distillation and connect the flask to the distillation apparatus (see sketch). Fill the dropping funnel with 50 mL potassium permanganate solution (Note 4). Place 10 mL of sodium bisulfite solution in a 125 mL Erlenmeyer receiving flask and attach to the condenser in such a manner that the delivery tube is submerged in the solution (Note 5). Heat the boiling flask so that boiling begins in about 3 mins. When boiling begins, add permanganate solution dropwise at a rate such that 40 mL ( $\pm 5$  mL) will be added in 15 mins. while the boiling continues. Note the time. After the oxidation and distillation have proceeded for 13 mins., lower the receiver so that subsequent distillate will rinse down the tube of the condenser. After an additional 2 mins. (total reaction time 15 mins.), discontinue the addition of permanganate and remove the heat source (Note 6). Wash the tip of the condenser tube with a fine stream of purified water into the receiving flask. The total volume of distillate and washings should not exceed 75 mL.

Add 2 mL of starch indicator solution to the receiver and place it in an ice bath for about 5 mins. (Note 7). Remove from ice bath, add 4% iodine solution until a deep blue color is obtained and discharge this color by dropwise addition of 0.1 *N* sodium thiosulfate solution. Wash down the walls of the receiver with a fine stream of purified water from a wash bottle and add carefully 0.01 *N* standard iodine solution until the blue starch-iodine end point is obtained (Note 8). Replace the flask in ice bath until ready for titration. Add 15 mL of saturated sodium bicarbonate solution and titrate the liberated sulfite with the standard 0.01 *N* iodine solution. As the consumption of iodine slows down, add 1 mL of 10% sodium

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carbonate solution and complete the titration. The blue end point should persist for at least 2 mins. (Note 9).

Conduct a blank determination on all reagents, substituting purified water for the sample and note the titer (Note 10).

**CALCULATION**

$$\% \text{ Lactic Acid (as is)} = \frac{(\text{mL } 0.01 \text{ N I}_2 - \text{Blank})(0.45) * (200 \text{ mL})(200 \text{ mL})(100)}{(1000)(25 \text{ mL})(\text{Sample Wt.,g})}$$

\*1 mL of 0.01 *N* iodine solution is equivalent to 0.45 mg of lactic acid.

**NOTES AND PRECAUTIONS**

1. The steeping (soaking) of corn in a dilute sulfurous acid solution extracts solubles including carbohydrates and the latter are largely fermented to lactic acid during the process. The concentrated extract is known as heavy steepwater in the corn wet milling industry and it is designated Condensed Fermented Corn Extractives by the American Association of Feed Control Officials. The latter organization provides the following definition:

"Condensed Fermented Corn Extractives is the product obtained by the partial removal of water from the liquid resulting from steeping corn in a water and sulfur dioxide solution which is allowed to ferment by the action of naturally occurring lactic acid producing microorganisms as practiced in the wet milling of corn."

2. The oxidation-distillation technique is not specific for lactic acid. Carbohydrates seem to be the principal interfering substances in steepwater and these are removed by treatment with copper sulfate and calcium hydroxide.

The procedure determines free lactic acid and its salts. Lactides and some lactate esters will be included in the result if the sample is saponified for 30 mins. in 1 *N* sodium hydroxide at *room temperature* prior to oxidation and

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distillation. Saponification at elevated temperatures must be avoided because alkali degradation of carbohydrates yields lactic acid.

3. Commercial (heavy) steepwater normally contains about 50% dry substance; proportionately larger samples should be taken at lower dry substance levels.
4. Manganous ions react with permanganate to form manganic ions which effect the oxidation of lactic acid to acetaldehyde. The excess of manganous sulfate prevents the oxidation of acetaldehyde to acetic acid by permanganate.
5. The amount of bisulfite in the receiving flask is a large excess to prevent loss of acetaldehyde.
6. Distillation of acetaldehyde is rapid and is usually complete in less than 10 mins.
7. Cooling in an ice bath prior to removal of excess bisulfite and titration retards air-oxidation of liberated bisulfite and sharpens the end point.
8. Addition of 4% iodine solution removes the excess bisulfite. Final solution adjustment prior to titration requires careful addition of 0.1 *N* sodium thiosulfate solution and 0.01 *N* iodine solution. Improper excesses of either thiosulfate or iodine will affect the final titer.
9. After addition of sodium bicarbonate solution, titration with 0.01 *N* iodine solution should be performed rapidly to prevent air-oxidation of sulfite. Carbonate addition should be made only near the end point; a second addition of carbonate solution can be made to determine whether or not a true end point has been obtained. Excesses of sodium carbonate must be avoided to prevent air-oxidation of sulfite and to prevent extraneous consumption of standard iodine solution.
10. Blank determinations rarely produce titers equivalent to more than 0.1 mL of 0.01 *N* standard iodine solution. Higher blank titers are seldom

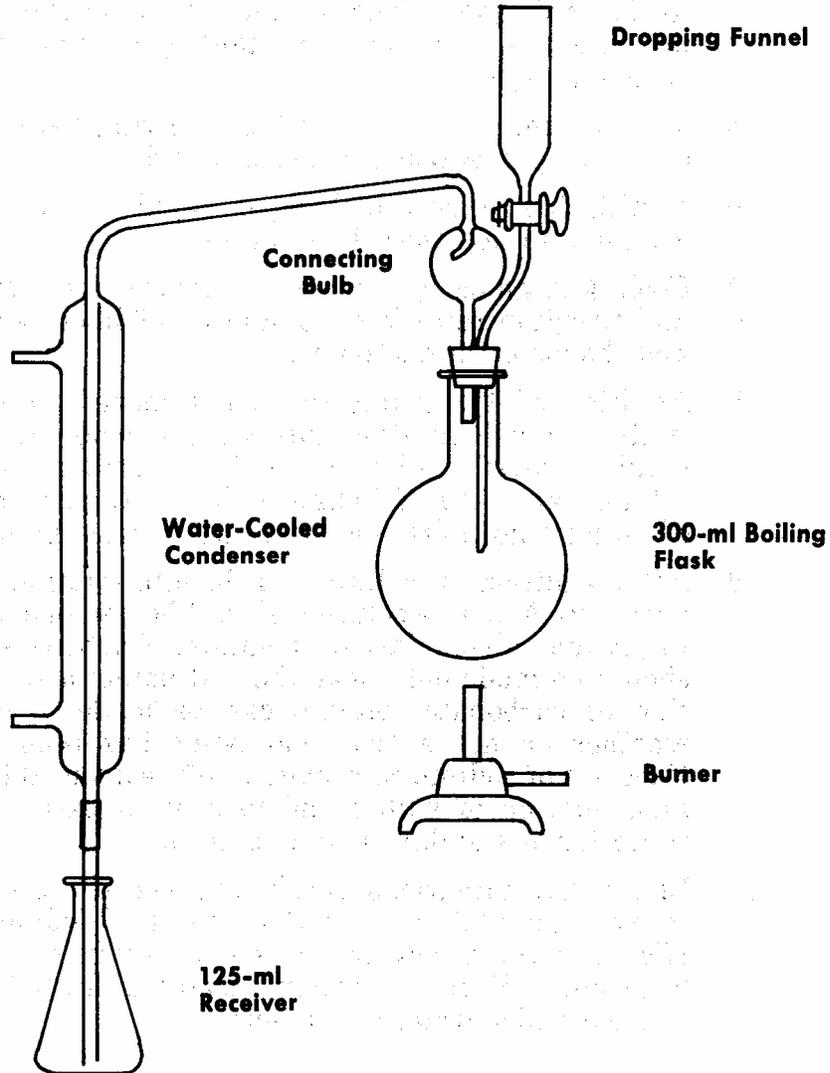
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reproducible and must be avoided. High blank titers usually indicate impurities in the purified water or reagents.

**METHOD HISTORY**

Steepwater, Lactic Acid (J-36), Date of Acceptance 11-12-1962, Revised 3-10-1997.

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Oxidation-Distillation Assembly