

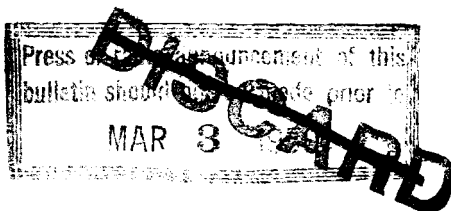
FEB 27 1950

A Test for
Quaternary Ammonium
Compounds *in*
Milk *and in*
Detergent Sanitizers

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Circular of Information 472

January 1950

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The use of quaternary ammonium compounds for a number of sanitizing operations in the dairy industry has resulted in a need for a simple, sensitive method for determining concentration of these compounds in milk. The following method, which has been developed for this purpose, will determine the concentration of the commonly used commercial quaternary ammonium compounds when they are present in quantities of 5 parts per million (ppm) or more in milk. The first six steps of the procedure may be employed as a presumptive test to detect presence of quaternaries in milk. The remaining steps should be carried out for an accurate determination of the total quantity present in the sample.

Reagents

1. Indicator Solution

- a. Dissolve eosin yellowish dye (dye concentration of about 90 per cent) in acetone (analytical grade) at the rate of 0.5 mg. of dye to 1 ml. of acetone.
- b. Add acetone-eosin solution to tetrachloroethane at the rate of 1 ml. of acetone-eosin to 9 ml. of tetrachloroethane.
- c. Remove the reddish color from the solution by adding citric acid crystals (analytical grade) at the rate of 10 mg. of crystals to each ml. of dye solution.
- d. Shake for one minute or until the solution becomes colorless.
- e. Filter through filter paper.

2. Buffer

Prepare a 25 per cent solution of citric acid (analytical grade) and adjust to pH of 3.5 with 50 per cent sodium hydroxide. Approximately 12 ml. of sodium hydroxide to 100 ml. of citric acid usually are required.

3. Anionic Solution

Prepare a 0.01 per cent solution of surface active anionic compound from 10 per cent Fisher Laboratory Aerosol (10 per cent di-octyl sodium sulfo-succinate). This represents a 1:1000 dilution of the 10 per cent Aerosol. Other anionic surface active agents may prove suitable for this solution. The anionic solution can be standardized against a quaternary solution of known concentration.

Procedure

1. Place 1 ml. of the milk to be tested in a test tube.
2. Add 5 ml. of distilled water.
3. Add 1 ml. of dye solution.
4. Add 0.2 ml. of buffer solution.
5. Plug the test tube and shake vigorously for 10 seconds.
6. Centrifuge until three distinct layers appear. The center layer of precipitated protein at this stage should be packed firmly. The period required may be about 5 minutes for a 10-inch centrifuge operated at 3,400 rpm. A Babcock centrifuge may be used but may require as much as 25 minutes for this operation. The top layer should be a clear liquid, the middle layer should consist chiefly of precipitated protein, and the lower layer should consist of the solvent--containing approximately 80 per cent of the quaternary ammonium compound. Presence of a quaternary ammonium compound is indicated by development of a red color in the solvent layer. The color may be intensified by subsequent steps in the procedure.
7. Remove the top layer of clear liquid by suction. Pour out the lower layer of solvent into a clean test tube. The precipitated protein will adhere to the first tube when the solvent layer is transferred.
8. Add 5 ml. of distilled water to the solvent layer in the second tube and agitate for about 5 seconds in such a manner that the contents are thoroughly mixed. If the agitation is too vigorous at this stage, an emulsion will be formed and difficulty will be encountered in obtaining separation into water and solvent layers.
9. Allow all of the solvent to settle to the bottom of the tube and remove the top layer with suction. The rate of separation of the solvent from the water layer can be accelerated by centrifuging for about 20 seconds.
10. Repeat this washing and separation twice more.

11. Add 5 ml. of distilled water and 0.1 ml. of buffer to the solvent layer after the third washing.
12. Titrate this mixture with anionic solution. Add slowly and shake until the red color of the lower layer fades out and it becomes clear. The quantity of quaternary ammonium compound is indicated by the quantity of anionic compound required to remove the red color from the lower layer. This quantity in ml. multiplied by the factor 125 yields the concentration of quaternary ammonium compound in ppm (plus or minus 2 ppm). A micro-burette facilitates accurate titration.
13. The total quaternary ammonium compound present in the original sample may be recovered and titrated by saving the water layers removed in steps 7, 8, 9, and 10 and repeating the test from steps 3 to 12, inclusive, on this composite. This procedure must be repeated until all quaternary ammonium compound has been extracted. Usually 3 or 4 separate extractions are required to recover all of the quaternary when 100 ppm are present in the milk.

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The foregoing procedure, with a slight modification, also can be employed as an all-purpose method to determine concentration of quaternary ammonium compounds in the form of a water solution of the unbuffered quaternary alone or in the form of buffered quaternary, fortified quaternary or detergent sanitizer solutions. The modification involves preparation of the indicator solution with 0.1 mg. of eosin yellowish dye per ml. of acetone rather than 0.5 mg. of dye. A fourth washing may be necessary to remove interfering factors in determining quaternary concentration in solutions of detergent sanitizer. If concentrations of unbuffered or unfortified quaternaries alone in water solution are to be determined, one drop of buffer rather than 0.2 ml. is sufficient and the contents of the tube may be titrated without washing.

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Acknowledgment

This work was supported in part by a grant from Klenzade Products, Inc.