REPORT ON HYDROGEN PEROXIDE IN MILK

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Hydrogen peroxide has been used for many years as a preservative for laboratory samples of milk and cream. It is possible that it could be used as a preservative for market milk; if so, the milk would be rendered adulterated under present food laws. Thus it is desirable to have an official method for its detection.

The Associate Referee has surveyed numerous methods for the detection of hydrogen peroxide in aqueous solutions to determine if any of these methods can be adapted for use with milk. Of the methods tested, the most sensitive and easiest to use was the Arnold-Mantzel method (*The Merck Index*, 5th Ed.). According to this method, when the proper amount of a vanadic acid reagent solution is added to liquids containing hydrogen peroxide, a permanent red color appears. The method is sensitive to 0.0006 per cent.

A solution of vanadic acid was prepared by dissolving 1 gram of vanadium pentoxide in 100 ml of 10 per cent sulfuric acid. When 1–20 drops of this solution was added to milk containing hydrogen peroxide, a light pink to dark brick red color was formed in the milk, depending on the concentration of hydrogen peroxide present. In milk containing a high concentration of hydrogen peroxide (more than 1000 ppm) the color "bleached out." However, when more vanadic acid solution was added, the color reappeared. This color was stable; it lasted from 1 to 8 hours depending on the type of milk, the concentration of hydrogen peroxide, and the amount of vanadic acid reagent used.

Raw and pasteurized milk were both tested with the vanadic acid reagent. The test proved equally sensitive in both types of milk; however, the hydrogen peroxide is not as stable in the raw milk.

This test was used to determine the stability of hydrogen peroxide in milk. Results are summarized in Table 1.

On the basis of the findings shown in Table 1, a collaborative study was made of the vanadic acid method. Because of the instability of hydrogen peroxide, prepared samples were not sent to the collaborators; instead they were instructed to obtain their own samples.

INSTRUCTIONS TO COLLABORATORS

PREPARATION OF SOLUTIONS

(a) *Milk.*—Sweet milk (pasteurized and raw) known to be free of hydrogen peroxide (local retail milk was found to be satisfactory in the Minneapolis area).

(b) *Hydrogen peroxide.*—3% H_2O_2. Analyze to find actual H_2O_2 content (U.S.P. XV method is satisfactory). *Note:* If only 30% H_2O_2 is available, dilute to 3% before adding to the milk.

TABLE 1.—*Stability of hydrogen peroxide in milk at various concentrations*

H_2O_2 PRESENT, PPM	HOURS AFTER PREPARATION										
	0	1	4	8	16	24	48	72	96	120	168
Raw Milk											
10	−	−	−	−	−	−	−	−	−	−	−
100	+	+	+	+	−	−	−	−	−	−	−
1,000	+	+	+	+	+	+	+	−	−	−	−
10,000	+	+	+	+	+	+	+	+	+	+	+
Pasteurized Milk											
10	−	−	−	−	−	−	−	−	−	−	−
100	+	+	+	+	+	+	+	+	−	−	−
1,000	+	+	+	+	+	+	+	+	+	+	−
10,000	+	+	+	+	+	+	+	+	+	+	+

(c) *Vanadic acid reagent.*—Dissolve 1 g vanadium pentoxide in 100 ml dilute H_2SO_4.

PREPARATION OF H_2O_2-TREATED MILK

Prepare two series of samples, using both pasteurized and raw milk. Add a sufficient volume of the analyzed 3% H_2O_2 solution to a 100 ml volumetric flask so that exactly 0.1 g H_2O_2 is present, and dilute to volume with sweet milk (Solution 1, 0.001 g/ml, 1000 ppm). Pipet 10 ml of Solution 1 into a second 100 ml volumetric flask and dilute to volume with milk (Solution 2, 0.0001 g/ml, 100 ppm). In same manner, use 10 ml Solution 2 to prepare Solution 3 (0.00001 g/ml, 10 ppm) and 10 ml Solution 3 to prepare Solution 4 (0.000001 g/ml, 1 ppm).

DETECTION OF HYDROGEN PEROXIDE

Prepare a series of 5 clean test tubes of convenient size (20–30 ml). To one, add 10 ml untreated milk for the blank; to each of the others, add, respectively, 10 ml each of Solutions 4, 3, 2, and 1. To each tube add ca 10 drops of vanadic acid reagent and give the tube a quick, hard shake to mix (the acid causes the milk to curdle); then observe the tubes for development of orange-pink to brick-red color (color develops almost immediately). If red color bleaches out, due to high concentrations of H_2O_2, add more vanadic acid reagent to restore color. Note the lowest concentration of H_2O_2 that develops pink color.

Let tubes stand overnight and again examine, this time for greenish-yellow color in the curd layer. Note the lowest concentration of H_2O_2 in which this color is observed. Determine sensitivity as follows:

Prepare fresh solutions of milk (pasteurized and raw) comparable to the lowest concentration of H_2O_2 that gave a positive test in the first series. From this solution prepare second series of dilutions in steps of 10 ppm by pipetting (*a*) 1 ml into a test tube and diluting to 10 ml; (*b*) 2 ml in the second test tube and diluting to 10 ml, etc., up to 10 tubes. Test as before. Again let tubes stand overnight and examine for the yellow color in curd layer.

Example: If a pink color is detected in Solution 2 but not in Solution 3, H_2O_2 is detectable in concentrations of 10–100 ppm. To determine the lowest detectable

concentration, test concentrations of less than 100 ppm but not less than 10 ppm, by preparing blank tube, No. 1, and other tubes as follows:

To tube No. 2, add 1 ml Solution 3 and 9 ml milk (10 ppm); to tube No. 3, add 2 ml Solution 3 and 8 ml milk (20 ppm); to tube No. 4 add 3 ml Solution 3 and 7 ml milk (30 ppm), etc., up to 100 ppm.

Report the lowest concentrations of H_2O_2 detected by both techniques and describe the colors observed.

RESULTS AND DISCUSSION

Table 2 lists the lowest concentrations at which a positive test for hydrogen peroxide was obtained by the Associate Referee and the collaborators.

TABLE 2.—*Collaborative results for lowest concentration of hydrogen peroxide detected in milk*

COLLABORATOR	H_2O_2 CONCENTRATION, PPM	
	RAW MILK	PASTEURIZED MILK
Associate Referee	30	30
A	20	20
B	50	50
C	70	60
D	80	30
E	100[a]	70

[a] Not reported between 10–100 ppm.

The alternate technique of letting the milk stand overnight with the vanadic acid reagent did not substantiate the Associate Referee's findings. Table 3 lists the results obtained by using the alternate procedure.

Hydrogen peroxide was detected in milk serum from hydrogen peroxide-treated milk by the vanadic acid reagent. The test was found to be even more sensitive for milk serum than for whole milk (to less than 10 ppm).

TABLE 3.—*Collaborative results by alternate method*

COLLABORATOR	H_2O_2 CONCENTRATION, PPM	
	RAW MILK	PASTEURIZED MILK
Associate Referee	10	10
A	20	30
B	50	50
C	100	100
D	No color change	1000
E	No color change	No color change

A spectrophotometric curve was made from milk serum containing 10 ppm hydrogen peroxide. Peak absorbance was found at 460 mμ. A curve

was prepared by plotting concentration of hydrogen peroxide from 0 to 700 mg per 25 ml of milk serum *versus* absorbance at 460 mμ. Essentially a straight line was obtained. Because of the difficulty of obtaining the milk serum free of interfering substances, the quantitative procedure was not pursued further, since the problem was primarily qualitative.

SUMMARY AND RECOMMENDATION

When a 1 per cent solution of vanadic acid is added to milk containing hydrogen peroxide, a pink to brick-red color is produced. The color developed by the vanadic acid reagent is sensitive to less than 80 ppm hydrogen peroxide in milk. Hydrogen peroxide decomposes rapidly in milk; this decomposition is faster in raw milk than in pasteurized milk.

If hydrogen peroxide has been added to milk in concentrations of 300–1000 ppm (1–4), it can be detected by the vanadic acid reagent 16–72 hours after its addition. Vanadic acid reagent also offers a means of quantitative measurement of hydrogen peroxide in milk when samples of clarified milk serum are used.

It is recommended* that the following qualitative test for the detection of hydrogen peroxide in milk be adopted as first action:

(a) *Reagent.*—Dissolve 1 g V_2O_5 in 100 ml H_2SO_4 (6+94).

(b) *Test.*—Add 10–20 drops reagent to ca 10 ml sample and mix. Pink or red color indicates H_2O_2.

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No reports were given on benzoates and hydroxybenzoates, benzoates in meats, boric acid in meats, dimethyldichlorosuccinate and dehydroacetic acid, fluorides, monochloroacetic acid, quaternary ammonium compounds, radiation preservation of foods, sorbic acid, or thiourea.

* For report of Subcommittee D and action of the Association, see *This Journal*, 40, 37, 38 (1957).