Analysis of Hydrogen Peroxide in Milk Using Titanium Tetrachloride

Abstract

Two to 3 μg hydrogen peroxide per milliliter can be detected in milk using titanium tetrachloride, if the proteins are precipitated with 1% trichloroacetic acid. The clear milk–trichloroacetic acid filtrate is treated with titanium tetrachloride and the absorbance measured at 415 nm. Absorbance varies linearly to at least 150 μg hydrogen peroxide per milliliter.

Introduction

Gilliland (2) recently introduced an enzymic method for analysis of hydrogen peroxide in milk. His method is over 30 times more sensitive than the titanium tetrachoride method of Amin and Olson (1). We have found that the sensitivity of the titanium tetrachloride method can be increased by precipitating the milk proteins with more dilute trichloroacetic acid. Our method is nearly as sensitive as Gilliland's and is faster than either the enzymic or earlier titanium tetrachloride analyses.

Method

All reagents were analytical grade. Homogenized whole milk (University dairy plant) was heated to 80 C for 5 minutes to destroy residual enzymic activity. Hydrogen peroxide (Baker Chemical Co.) was standardized using potassium iodide and sodium thiosulfate. Standards were prepared by adding 1 ml of water containing the appropriate amount of hydrogen peroxide to 9 ml of heated milk. Titanium tetrachloride (4 mg/ml) was prepared according to Wolfe (3).

Four milliliters of milk containing hydrogen peroxide were added to 4 ml of 2% trichloroacetic acid, well-mixed with a glass rod, and filtered through Whatman no. 2 filter paper. A 2.5-ml aliquot of the filtrate was added to 1 ml of titanium tetrachloride solution and the absorbance measured against a reagent blank (filtrate + $TiCl_4$) at 415 nm on a Beckman DU-2 spectrophotometer. Three series of standards containing 0 to 100 μg hydrogen peroxide per milliliter were analyzed in duplicate.

Results and Discussion

In the method of Amin and Olson (1), milk proteins from 3 ml of milk were precipitated with 2 ml of 70% trichloroacetic acid and the mixture diluted to 100 ml to obtain a clear filtrate. In preliminary tests we found that a clear filtrate could be obtained by precipitating milk proteins with either 0.6 to 1% or 7.5 to 10% trichloroacetic acid, obviating the need for dilution (Table 1); other concentrations of the acid gave turbid filtrates. When clear filtrates were mixed with titanium tetrachloride, turbidity developed after a few hours except when 10% trichloroacetic acid was used (Table 1). Development of turbidity in the presence of titanium tetrachloride was delayed for more than 12 hours if the clear filtrate was diluted even a small amount, such as 2 ml filtrate and 0.5 ml water.

An approximately twofold dilution of the milk with trichloroacetic acid is apparently necessary to obtain a clear filtrate. For example, 5 ml milk and 2.5 ml of 3% trichloroacetic acid yielded a turbid filtrate, even though the final acid concentration was 1%. Thus, a 9:1 milk-

TABLE 1. Turbidity of trichloroacetic acid–milk filtrates and their stability to titanium tetrachloride.

Trichloroacetic acid (%)	Turbidity of filtrate	Stability to $TiCl_4$
0.5	Slightly turbid	Turbid in 1 hour
0.6	Clear	Turbid in 2 hours
1.0	Clear	Turbid in 2.5 hours
2.5	Turbid	.....
5.0	Turbid	.....
7.5	Clear	Turbid in 3 hours
10	Clear	Clear for > 24 hours
25	Turbid	.....

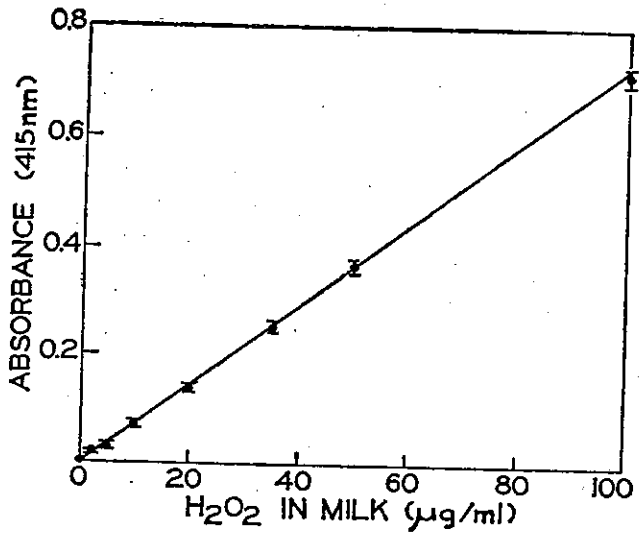

FIG. 1. Standard curve for hydrogen peroxide in milk determined by treating a milk–trichloroacetic acid filtrate with titanium tetrachloride. Points indicate means; brackets indicate standard deviations.

water mixture used to prepare the standard curve in Figure 1 may have prevented turbidity. However, we have routinely analyzed milk containing only 2.5 ml hydrogen peroxide per 100 ml milk with no apparent adverse effects. If turbidity develops in unknown samples, addition of 1 ml of water per 9 ml of milk should prevent turbidity.

Initially a standard curve was made with 0.6% trichloroacetic acid, but reproducibility was poor and the curve was linear only to about 40 µg hydrogen peroxide per milliliter. When a final concentration of 1 or 10% trichloroacetic acid was used, the curve was linear to at least 150 µg hydrogen peroxide per milliliter. The detection limit of the method is 2 to 3 µg hydrogen peroxide per milliliter of milk.

Acknowledgments

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L. K. FERRIER, N. F. OLSON, and T. RICHARDSON, Department of Food Science, University of Wisconsin, Madison 53706

References

(1) Amin, V. M. and N. F. Olson. 1967. Spectrophotometric determination of hydrogen peroxide in milk. J. Dairy Sci., 50: 461.

(2) Gilliland, S. E. 1969. Enzymic determination of hydrogen peroxide in milk. J. Dairy Sci., 52: 321.

(3) Wolfe, W. C. 1962. Spectrophotometric determination of hydroperoxide in diethyl ether. Anal. Chem., 34: 1328.

Simplified Pouch Method for Enumeration of "Stinker" Swiss Cheese Organisms [1]

Abstract

Clostridium species have been successfully enumerated in a rectangular, commercially available boil-in-bag pouch. One milliliter of raw milk and 45 ml of Fe^{++}-containing dextrose-free thioglycollate medium are sealed in the pouch, heated for 10 minutes at 80 C, and allowed to solidify. Hydrogen sulfide–producing colonies are enumerated after 48 hours at 37 C.

Introduction

The lack of simple anaerobic culturing techniques has prevented routine screening of raw milk samples for species of *Clostridium* implicated in some Swiss cheese defects. Recently, Hettinga, Vedamuthu, and Reinbold (5) described the use of an oxygen-impermeable, round plastic pouch for culturing *Propionibacterium*. This pouch was first described by Bladel and Greenberg (2) for *Clostridium* studies. Bladel (1) has since been issued a patent covering the round pouch. This paper describes an attempt to adapt a commercially available rectangular plastic pouch for tracing Swiss cheese "stinker" organisms to their source.

Previous workers have traced the stinker defect to hydrogen sulfide–producing *Clostridium lentoputrescens* (4), which occurs in milk as do other *Clostridium* species implicated in gas blowing (7). The organisms grow in certain ensilage products then, following passage through the cow, contaminate milk via dust and manure. Several workers have reported control of spore formers through milk treatment (6, 9–11). The variable results reported, the problems with practical application of the treatments, and the relationship of the problem to

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