

Influence of Storage, Heat, and Homogenization upon Xanthine Oxidase Activity of Milk

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ABSTRACT

Xanthine oxidase activity in milk was determined by measuring the rate of formation of vanillic acid from vanillin. Raw milk received at a dairy plant had .208 units xanthine oxidase activity per ml and after 24-h storage at 4 C, .228 units per ml. Upon further storage activity decreased. Heating the fresh raw milk in a water bath to 55 C increased xanthine oxidase activity to .236 units per ml. Partial inactivation of the enzyme occurred when milk was heated at 60, 65, or 70 C for 5 min, and destruction was almost complete with heat at 75 C for 5 min. Raw milk heated at 48 C for 5 min and homogenized at pressures between 70.3 and 281.2 kg/cm² had xanthine oxidase activities which were a linear function of pressure and showed that each additional kg/cm² pressure resulted in additional xanthine oxidase activity of .16 milliunits per ml of milk.

INTRODUCTION

Raw bovine milk is a rich source of the enzyme xanthine oxidase (EC 1.2.3.2) (1). According to one hypothesis (7, 8), homogenization of milk causes the enzyme to become more biologically available, enabling it to enter the blood stream and become causative factor in atherosclerotic lesions.

Gudnason and Shipe (4) found an increase in apparent xanthine oxidase activity as raw milk was stored at 4 C. The activity reached a maximum after 24-h storage. They also found that homogenization at unspecified pressure increased enzyme activity. Cerbulis and Farrell (2), who recently reported the xanthine oxidase activity of milk in various stages of processing, found that raw milk as received at the commer-

cial plants contained about .1 unit per ml of milk.

Our purpose was to determine the influence of storage, heat treatment, and homogenization pressures upon xanthine oxidase activity in milk.

MATERIALS AND METHODS

Source of Raw Milk

Raw milk samples were obtained from the storage tank at the University of Tennessee Dairy Plant at approximately 0800 on the day of the experiment. Such milk was a mixture of the two previous milkings of the University Dairy Herd. Milk for storage studies was protected from light by shielding it with brown paper over glass containers.

Standard Curve

Xanthine oxidase activity was determined by the method of Kuramoto et al. (5). Xanthine oxidase, extracted from milk and suspended in .6 saturated ammonium sulfate solution, was purchased from ICN Pharmaceuticals, Inc., Cleveland, OH. After dilution of the suspension of enzyme with water, aliquots were added to 10 ml boiled milk to give concentrations of 0 to .080275 units per ml. A unit of xanthine oxidase activity is defined as that amount forming one micromole of uric acid per min at 25 C (9). The relationship between xanthine oxidase activity and absorbance values at 650 nm was:

$$Y = -.0058 + .5431X$$

where Y = xanthine oxidase activity per ml, and X = absorbance. The correlation coefficient was .997.

EXPERIMENTAL TREATMENTS

Xanthine oxidase activity was determined on raw milk within 2 h after securing the sample and after 24, 48, 72, and 96-h storage at 4 C.

Received August 23, 1977.

Raw milk was heated to 55, 60, 65, 70, or 75 C for 5 min and cooled to 4 C. Xanthine oxidase activity was determined within 2 h and after 24 h storage at 4 C.

Raw milk was heated to 48 C for 5 min, homogenized in a single stage Gaulin homogenizer at 70.3, 105.4, 140.6, 175.7, 210.9, 246.1, or 281.2 kg/cm², and cooled to 4 C. Xanthine oxidase activity was determined within 2 h. Samples homogenized at 175.7, 210.9, 246.1, and 281.2 kg/cm² also were analyzed for xanthine oxidase activity after 24, 48, 72, or 96 h at 4 C.

STATISTICAL ANALYSIS

The xanthine oxidase activities in units per ml of milk were the data points for the analysis of variance. Differences among treatment effects were partitioned by orthogonal polynomials. High order terms were included in the model if the partial regression coefficient was significantly different from zero.

RESULTS AND DISCUSSION

Storage Duration

The activity of xanthine oxidase in raw milk increased in 24 h at 4 C but decreased thereafter (Fig. 1). Regression analysis of activity against time yielded a quartic function represented by the equation:

$$Y = .2085 + .0239X - .0092X^2 + .0012X^3 - .00005X^4$$

where Y = xanthine oxidase activity in units per

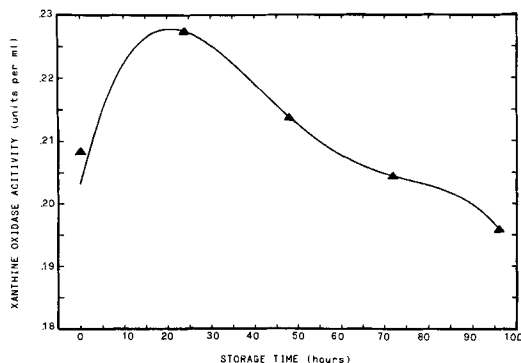


FIG. 1. The influence of storage at 4 C on the xanthine oxidase activity of raw milk (means of eight trials).

ml, and X = storage time in hours.

This increase in activity at the end of 24-h storage is in keeping with earlier results (4). A large part of the enzyme is in the fat globule membrane (6). Since the enzyme may become detached from the fat globule during storage for 24 h and become more accessible to the substrate. After this storage, other unknown factors apparently decrease the enzyme activity.

Heat Treatment

The average xanthine oxidase activity of eight samples of raw milk as received at the processing plant was .208 ± .0043 units per ml, and after it was heated to 55 C for 5 min the activity was .236 ± .021, a difference which was significant at .01.

Although 55 C is considerably below pasteurization temperatures, it is the approximate temperature at which milk comes from the regenerator section of the high-temperature-short-time pasteurizer. Previous research has shown increases in xanthine oxidase activity by heat treating milk to as high as 70 C for 5 min (4).

The enzyme was inactivated partially by heat treatment at 60, 65, and 70 C and almost completely inactivated when the milk was heated at 75 C for 5 min (Fig. 2). The influence of storage (2 h vs. 24 h) of the heated milk was not significant (P>.05); therefore, the data for both storage times were treated as one effect and resulted in the regression equation:

$$Y = 17.7139 - .8619X + .0141X^2 - .00008X^3$$

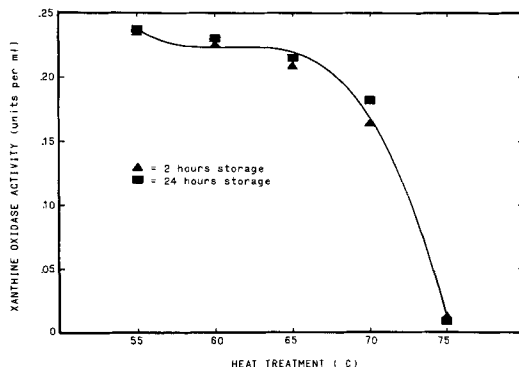


FIG. 2. Influence of heat treatment and storage at 4 C on the activity of xanthine oxidase of milk (means of eight trials for each storage duration).

where Y = xanthine oxidase activity in units per ml and X = temperature.

Homogenization Pressure

When the milk was analyzed within 2 h after homogenizing, the activity of xanthine oxidase increased as the pressure of homogenization was increased between 70.3 and 281.2 kg/cm², and the data yielded a linear function (Fig. 3) expressed by:

$$Y = .20905 + .0001605X$$

where Y = xanthine oxidase activity in units per ml, and X = homogenization pressure in kg/cm².

The correlation coefficient was .996. Each additional kg/cm² pressure of homogenization caused an increase in xanthine oxidase activity of .16 mU/ml of milk.

Homogenization Pressures and Storage

The xanthine oxidase activity of homogenized milk at 4 C increased with storage up to 24 h, but the activity decreased upon further storage of the milk (Fig. 4). Regression analysis of xanthine oxidase activity at different pressures over time yielded cubic functions represented by the equations:

$$\text{at } 175.7 \text{ kg/cm}^2, Y = .2389 + .0068X - .0019X^2 + .0001X^3$$

$$\text{at } 210.9 \text{ kg/cm}^2, Y = .2446 + .0069X - .0019X^2 + .00009X^3$$

$$\text{at } 246.1 \text{ kg/cm}^2, Y = .2518 + .0064X - .0018X^2 + .0001X^3$$

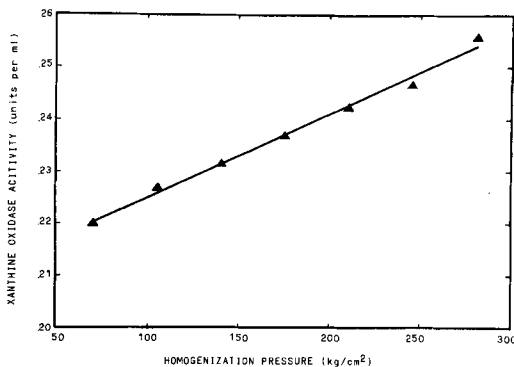


FIG. 3. Influence of homogenization pressure on the activity of xanthine oxidase of milk (means of six trials at each pressure).

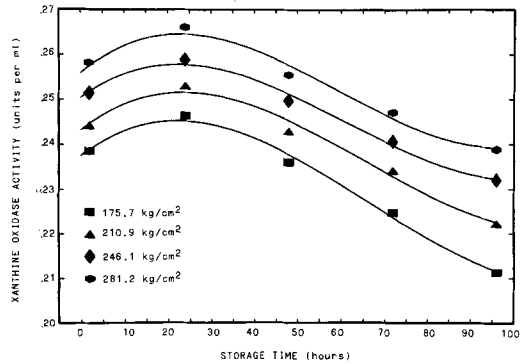


FIG. 4. Influence of homogenization pressure and storage on activity of xanthine oxidase of milk (means of six trials for each pressure).

$$\text{at } 281.2 \text{ kg/cm}^2, Y = .2585 + .0067X - .0020X^2 + .0001X^3$$

where Y = xanthine oxidase activity in units per ml milk and X = hours of storage.

The analysis of variance showed that milk homogenized at various pressures and stored for various times had significantly different xanthine oxidase activities ($P < .1$).

Homogenization and other mechanical stresses increase xanthine oxidase activity of milk (3, 4). The present investigation reveals a definite relationship between homogenization pressure and xanthine oxidase activity of raw milk previously heated to 48 C for 5 min. Since xanthine oxidase is not inactivated by this heat treatment, homogenization might be expected to release the enzymes from the fat globule membrane. Activation of lipase occurs under similar circumstances, at lower temperatures.

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