# DETECTION OF HEATED MILK ADMIXED WITH RAW MILK

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Milk that has been heated to temperatures below that of adequate pasteurization or pasteurized milk that has been mixed with raw milk to make it appear to meet the bacterial standards of Grade "A" raw milk for pasteurization is clearly not acceptable under the "Milk Ordinance and Code" *(8)*.

Numerous tests have been proposed for the detection of heated milk, and most of these have also been studied as tests for pasteurization. These include the peroxidase, lipase, amylase, vanillin, and phosphatase tests. All except the vanillin test are based upon measurement of the activity of a natural enzyme that occurs in milk. In the vanillin test the heated milk cannot be detected unless the heating has exceeded 80 C. This is also true in the peroxidase test. These tests would, therefore, appear to be of little value, for it is desirable to be able to detect milk heated to lower temperatures. The lipase test might be useful at lower temperatures, but no convenient method has been developed for measuring the lipase activity.

The amylase test was made the basis of a method for detecting heated milk by Scharer *(12)* in a procedure designed to yield colors more readily distinguishable than those produced in the phosphatase test. On further study of this method, it was found, as other investigators *(3, 9)* have indicated, that the amylase content of milk is too variable and that not enough is known about the amylases of milk, their temperature of inactivation, or their optimum pH. The type of starch used in such tests is also another variable that needs to be further studied.

Since the phosphatase test can detect as little as 0.05% raw milk and has been used by some to measure the phosphatase activity of raw milk, it now appears that the phosphatase test might be applicable to the detection of heated milk. As Scharer *(12)* proposed for the amylase test, by appropriate dilution of the suspected milk with boiled milk, the phosphatase test should be capable of detecting admixture of 25, 50 or 75% raw milk with pasteurized milk.

Assuming an average phosphatase activity of 2,500 μg of phenol per ml, a 25, 50, 75 or 100% raw whole milk diluted 1:250, 1:500, 1:750 and 1:1000 with boiled milk should have phosphatase activities as indicated in Table 1 when tested by the Scharer Laboratory Method.

On the same basis with the "Scharer Rapid Method," if the above four dilutions of a suspected milk are identified as A, B, C, and D respectively, then the sample is 25% raw milk or less if A is a light blue color and B a tint or no color. If A and B are colored but C is only a tint or has no color, the sample is 50% raw milk or less. If A, B, and C are colored but D is only a tint or has no color, the sample is 75% raw milk or less. If A, B, C, and D all show color, even though D has only a tint of color, the milk is probably completely raw milk. In determining the presence or absence of color, a control using a sample of boiled milk should always be run for comparison.

These procedures have been used on a number of occasions to detect the admixture of raw milk with laboratory pasteurized, as well as commercially pasteurized milk. The indications are that these procedures may also be used to detect milk that has been heated to less than pasteurization time and temperature. By referring to Figure 1, it can be seen that milk heated to 135 F for 30 min (which is sufficient to destroy *(5)* the lipase activity) will have approximately half of its normal phosphatase activity or about 1,000 to 1,200 μg/ml. If this milk is diluted with boiled milk as in the above test, it will test like a milk containing 50% raw milk. A milk heated to 155 F for 5 sec will test like a 25% raw milk, as will also a milk heated to 143 F for 2 min. A milk heated to 143 F for 20 min will have its phosphatase activity reduced to about that of a 1% raw milk and would have to be tested undiluted to be detected as underpasteurized.

Table 1. Phosphatase Activity of Diluted Milk in μg of Phenol Per ml

| Dilution | 25% | 50% | 75% | Raw |
|---|---|---|---|---|
| 1:250 | 2.5 | 5.0 | 7.5 | 10.0 |
| 1:500 | 1.2 | 2.5 | 3.7 | 5.0 |
| 1:750 | 0.8 | 1.7 | 2.5 | 3.3 |
| 1:1000 | 0.6 | 1.2 | 1.9 | 2.5 |

The curves in Figure 1 for temperatures of 155 F and 143 F were plotted from data published by Hetrick and Tracy *(6)*. The data plotted for the

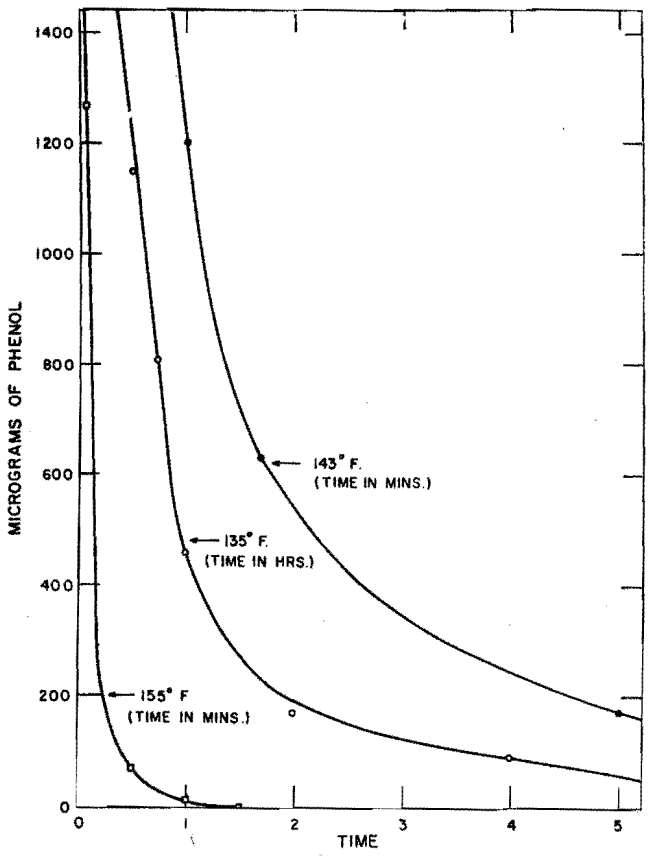


FIGURE I.—THERMAL INACTIVATION OF PHOSPHATASE.

inactivation of phosphatase at 135 F were obtained in this laboratory by heating samples of raw milk in a water bath for various periods of time. For those interested in a quantitative adaptation of the Scharer Laboratory Method and in carrying out such tests at other temperatures to judge the degree of heat treatment, the procedure is given below in detail.

## PROCEDURE

Samples of raw milk were heated in a water bath at 135 F for periods of 15, 30, and 45 min and for 1, 2, 3, 4, 5, and 6 hr. Forewarming for 5 min was required for the samples to reach a temperature of 135 F. This 5-min period of forewarming was not included as a part of the indicated holding times, for in comparison with the time required to inactivate the enzyme completely at 135 F, namely 6 hr *(6)* this forewarming time was relatively insignificant.

Phenol standards were prepared according to Scharer *(13)*. It has been found convenient for the purpose of preparing a standard curve to use standards containing 2, 5, 10, 15, 20, and 25 mg of phenol per 5 ml. Color was developed in the standards according to Scharer with allowances, for quantitative purposes, of at least 10 min for color development. Color was developed in ordinary laboratory test tubes (16 x 150-mm) and then poured into selected Coleman 12 x 75-mm cuvettes and read in a Coleman Jr. Spectrophotometer preferably within 30 min. A blank was prepared by adding to 5 ml of distilled water, all of the reagents, other than phenol, which are normally added to the phenol standards to develop a color. The instrument was zeroed on the blank at 100% transmission with the wavelength dial set at 610 m$\mu$. The standards and the blank may be extracted with 5 ml of butyl alcohol and the butyl alcohol extract used for preparing a standard curve.

In the latter case, the wavelength dial should be set at 650 m$\mu$. Wave lengths of 610 and 650 are the wavelengths of maximum absorption for the aqueous and butyl alcohol extracts, respectively, as shown in Figure 2, and must be used for maximum sensitivity. Butyl alcohol extraction is unnecessary in working with milk but may be desired or necessary in working with cheese. A separate standard curve is, however, required for each since standards extracted with butyl alcohol absorb to a greater extent even though a different wavelength is used (Figure 3).

Samples of milk for testing are prepared and color is developed as in the Scharer Laboratory Method *(13)*. A boiled milk sample is developed in the same manner and used as a blank for zeroing the instru-

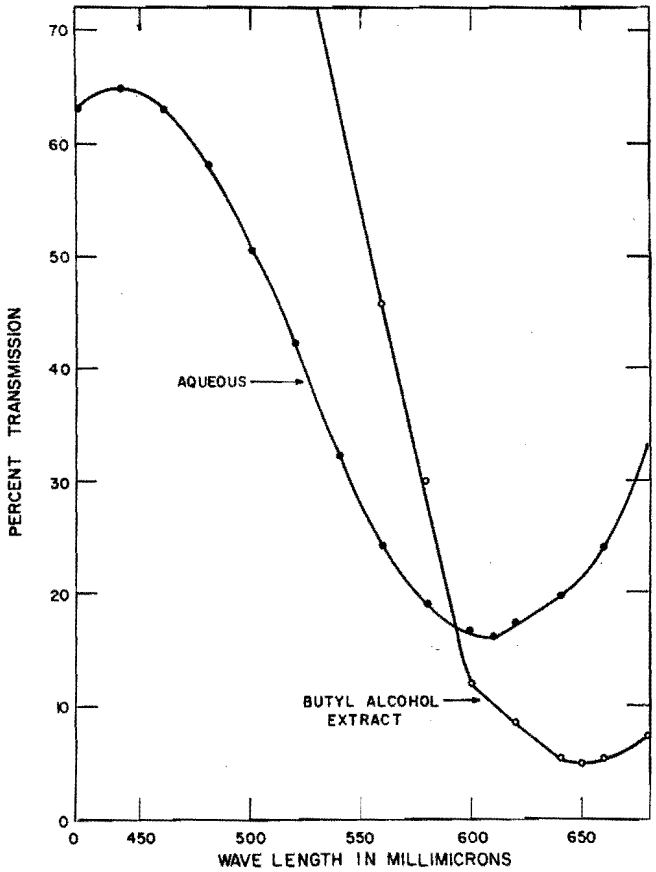


FIGURE II.—SPECTRAL TRANSMITTANCE CURVE FOR INDOPHENOL.

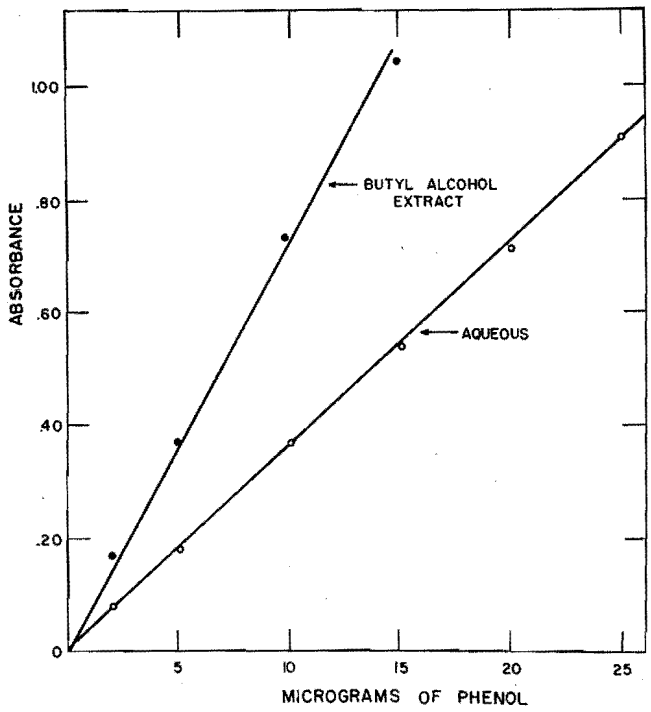


FIGURE III.—STANDARD CURVE FOR DETERMINATION OF PHOSPHATASE IN MILK AND CREAM.

ment at 100% transmission (0 absorbance). Concentration of the indophenol color developed in the milk samples is read from the standard curve prepared as above (Figure 3), and the results are multiplied by 2.3 to account for the dilution of the sample and to obtain the results in $\mu$g of phenol per ml of milk.

## DISCUSSION

As suggested above, the phosphatase content of milk would seem to be of rather constant value. As early as 1939, however, Burgwald *(2)* reported that milk from cows in early stages of lactation has a relatively low phosphatase activity and milk from mastitic udders usually has a relatively high phosphatase activity. Likewise, in 1939 Aschaffenburg and Neave *(1)* reported that milk in the early stages (10 to 60 days) of lactation was frequently found to be comparatively low in phosphatase activity, and that changes caused in milk by mastitis may increase the phosphatase activity in milk. Sanders *(11)* lists the phosphatase activity of milk obtained from several breeds of cows and from cows' udders that were infected with mastitis in some quarters. Sanders concludes that the phosphatase values are generally greater in Jersey (2,960 to 3,160 $\mu$g/ml) than in Holstein (620 to 800), and are strikingly higher in milk from mastitic udders (Jersey 4,500 and Holstein 1,750 to 2,200), and in severe mastitis (from 3,440 to 5,640 $\mu$g/ml). Haab and Smith *(4)* likewise found great variations in the phosphatase activity of individual cows, but also reported that during a complete year the phosphatase activity of pooled milk, representing approximately 500 cows, ranged only from 1,800 to 3,400 $\mu$g phenol per ml. Similarly, Hetrick and Tracy *(6)* reported that the phosphatase activity of ten lots of raw milk collected between February 20 and July 9, 1947, ranged only from 1,920 to 3,000 $\mu$g per ml with an average value of 2,230 $\mu$g of phenol per ml.

Actually, as indicated in Figure 4, by data obtained *(7)* from market milks or creams collected over a period of several months in Cincinnati, Ohio, the phosphatase activity varies with the fat content of the milk or cream and may be more directly related to the fat content than to either mastitis or the breed of the cow. In any case, all of these authors agree that the phosphatase activity is not likely to vary greatly in mixed herd milk, but obviously the phosphatase activity in any one cow's milk will make itself felt more and more as the number of animals whose milk is used for mixing decreases.

As is evident from Figure 4, Table 1 is applicable only in working with whole milk. If 30% cream were being tested to determine whether all or any part had been heated, a table like Table 1, but based on an average phosphatase activity of 8,000 $\mu$g of phenol per ml of 30% cream, would have to be constructed.

In making the determinations reported here, the raw milk was first diluted with boiled milk and then

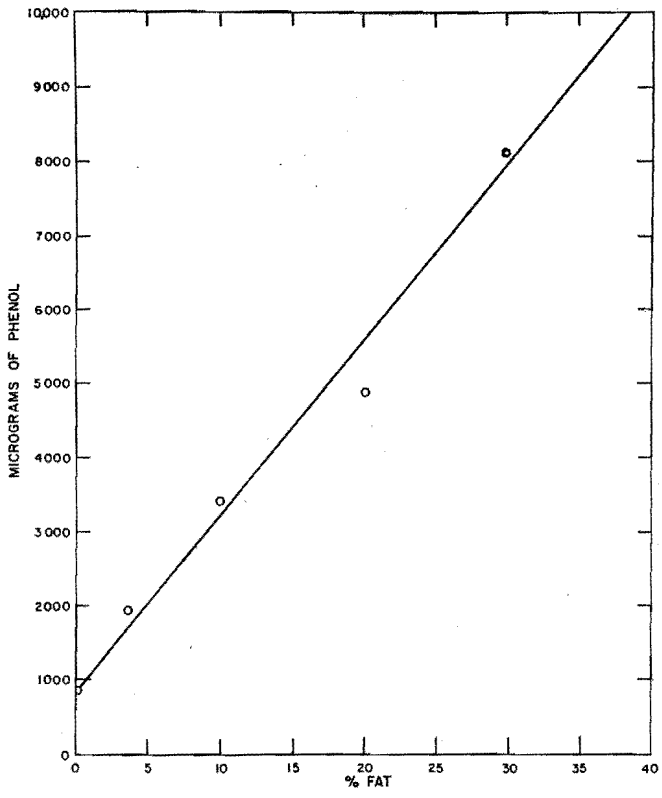


FIGURE IV.—RELATIONSHIP OF PHOSPHATASE ACTIVITY TO THE FAT CONTENT OF RAW MILK AND CREAM.

1 ml of this dilution was used in the regular phosphatase test. The amount of phosphatase activity was then measured quantitatively as outlined above. Alternate procedures would be to dilute the raw milk with buffer or water before or after color development or to use 2 g instead of 1 g of disodium phenylphosphate in the buffer substrate. These alternate procedures do not, however, give values as high or as consistent. Dilution with boiled milk is, therefore, preferred and is the procedure used also by Sanders and others.

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