The Laboratory Control of Added Water to Milk

A Discussion of the Cryoscopic Method

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THE increased demand for milk has prompted a few unscrupulous producers to add water to milk. Unfortunately, the routine milk control tests used in most public health laboratories are unable to detect this practice of adulteration. The usual milk control tests to detect adulteration, such as the Babcock tests for butterfat and lactometer readings for the specific gravity have been used in various formulae (i.e.,

$$\% \text{ added water} = 100 - \frac{\text{lactometer reading} - .8 \text{ of fat} \times 100)}{36}$$

to indicate the percentage of added water. This indirect method is unreliable and most often will not detect small amounts of added water. Also, it will not reveal adulteration by skimming and subsequent watering. On certain occasions a cow may give milk containing less than 8.0 per cent solids-not-fat; is the milk abnormal or watered? The analyst should resort to an accurate quantitative technique to answer that question; butter fat and total milk solids will not give sufficient information. Most mixed herds will yield a milk of which the butter fat and total solids values are above the standard requirements (i.e., 3.25 per cent and 11.25 per cent) and to variable extent (10 — 20 per cent) watering of the mixed milk can be tolerated without reducing butter fat and milk solids below the official figures.

However, there are approved quantitative techniques to determine watering. There are two official procedures for determining the quantity of added water, namely; by the use of an immersion refractometer to measure the refraction of the milk serum and by the use of the cryoscope to measure the freezing point of the milk. It was felt that a clarification of the standard and reliable cryoscopic method of testing for added water was needed to assist the many newcomers to the milk laboratory. This discussion is not intended to replace the official procedure as detailed in *Methods of Analysis* of the Association of Official Agricultural Chemists and the American Public Health Association's *Standard Methods of Milk Analysis;* its purpose is mainly to acquaint milk technicians with the principles and difficulties of the accurate determination of added water by this method.

The cryoscopic method for measuring added water to milk has been known for more than fifty years, but not until 1920 has an official agency in this country been concerned with this technique. In the early 1920's, a great deal of investigation [1] was carried out to standardize the appartus, procedures and interpretation of the freezing point determination of watered milk. The result of this work was the official approval by the Association of Official Agricultural Chemists of a standard procedure for the determination of added water to milk by the use of the Hortvet Cryoscope. With the improvement of milk quality in the past two decades, methods for measuring watering of milk fell into disuse. The dairy literature in American journals of the past five years has almost no references to watered milk and the cryoscopic technique. At the present time, the need for a reliable, simple method to determine added water has been emphasized by two factors; namely, the increase in watering induced by milk shortages, and secondly, by the increase in educational efforts to sell milk as a nearly perfect food. Any adulteration of a food will gradually bring that food into ill repute and thus vastly reduce its significance in a nutrition program.

PRINCIPLE OF METHOD

Normal whole milk, regardless of butter fat and solids content, has a constant freezing point which is defined as the temperature at which the liquid begins to crystallize out of solution. The freezing point of normal milk depends primarly upon the osmotic pressure of the secretions of the animal; this osmotic pressure is a constant value in milk.

If there is a deficiency in milk sugar, the metabolic processes of the animal causes an equivalent increase in another constituent, usually sodium chloride, which readjusts the osmotic pressure of the milk and raises this pressure to the normal level.

Freezing points of pure substances are definite single points on a temperature scale. However, milk is a colloidal suspension of solids in a liquid and its freezing point is not a single value. Early work on the freezing point of normal milk gave the range of —0.529° C. to —0.566° C. The point —0.550° C. was accepted as the best expression of a single freezing point value and it is used in all calculations. Using this value, —0.550° C., the cryoscopic method has a maximum error of 3 per cent. Should you determine the freezing point of the milk of a particular herd, the method can detect as little as 0.5 per cent added water to the milk.

As milk is diluted with water its freezing point approaches that of water and depressions less than 0.550° C. will be observed. The addition of water to milk raises the freezing point; the temperature at which the mixture of milk and added water begins to freeze is higher than for whole, unadulterated milk. This is a simple statement but is a source of much confusion for the beginner inasmuch as the freezing point values are on the negative side of the temperature scale and raising of the freezing point is exhibited by a reduction in the whole number. The following table gives the freezing points of a few milk and water mixtures:

Substance	*Freezing point*
Pure milk	—0.550
5% added water to milk	—0.552
10% added water to milk	—0.495
20% added water to milk	—0.440
Pure water	0.000

Thus the only measurement necessary to determine added water is to find the freezing point of the sample. To make a freezing point test, an instrument is required to produce first a freezing temperature, and secondly to measure accurately the exact value of the freezing point. The first requirement is accomplished by the evaporation of ether (forcing air through ether) and the second requirement by a special sensitive thermometer having a range of only 3° C. divided into hundredths of a degree and which can be estimated to the nearest 0.001 of a degree. This thermometer is similar to a standard reagent used in analytical chemistry; it should be carefully and frequently calibrated to check it against a known thermometer maintained in the Bureau of Standards.

STANDARDIZATION OF THERMOMETER

The Bureau of Standards Thermometer gives the following results:

Substance	*Freezing point*	*Freezing pt. depression* (S—W)
Water (±W)	+0.079	....
7% sucrose (—S)	—0.343	—0.422
10% sucrose (—S)	—0.542	—0.621

The laboratory test thermometers have been very carefully manufactured and calibrated. But they are not perfect instruments and will vary with age, room temperature, humidity, and other uncontrollable factors. Consequently, they must be repeatedly standardized by comparison with the Bureau of Standards thermometer. This standardization or calibration requires the determination of three freezing point values, namely, that of recently boiled distilled water, 7 per cent sucrose, and 10 per cent sucrose. The detailed methods of operation of the cryoscope are not given in this paper; but standard texts, such as *Standard Methods for the Examination of Dairy Products*, 8th edition, 1941, American Public Health Association, and the *Official and Tentative Methods of Official Agricultural Chemists,* 5th edition, 1940, give the exact procedure.

Any deviation of the test thermometer from the perfect (Bureau of Standards) thermometer is corrected by a factor obtained as follows:

sample of milk, using thermometer # 1:

Freezing point ° C. of boiled water + 0.056.

Freezing point depression ° C of 7 per cent sucrose 0.425.

Freezing point ° C. milk sample — 0.458.

Correction factor of thermometer # 1 1.015.

The freezing point depressions are the algebraic difference between the freezing points of the recently boiled distilled water and the sample of milk, and the algebraic difference between the freezing points of the water and the sucrose. The word depression implies degrees below the freezing point of water (0 ° C.). Consequently, it is always a negative value even though it is expressed without the negative sign. For example, the freezing point depression of the 7 per cent sucrose solution using thermometer # 1 is: — 0.369 — (0.056) = 0.425 ° C. The freezing point depression of the milk sample is — 0.458 — (0.056) = 0.514 ° C. To obtain the corrected freezing point depression of the milk sample, it is necessary to adjust this value to the Bureau of Standards thermometer, as given by the following procedures:

1. Subtract freezing point depression of 7 per cent sucrose as determined by # 1 thermometer from the freezing point depression of the milk sample.
2. Multiply by correction factor.
3. Add freezing point depression of 7 per cent sucrose as determined by

Freezing Point Values Recorded

Thermometer	*Water*	*Freezing Point Depressions ° C.* 7% Sucrose	10% Sucrose	Interval
Bureau of Standards	+0.079	0.422	0.621	0.199
Test thermometer No. 1	+0.056	0.425	0.621	0.196
Test thermometer No. 2	+0.022	0.422	0.622	0.200

Correction factors:

No. 1 0.196 equivalent to 0.199
0.196X=0.199
X=1.015

No. 2 0.200X=0.199
X=0.995

Calculation of Results

The conversion of the freezing point obtained on the milk sample into the percentage of added water is a tricky calculation. The seventh edition (1939) of *Standard Methods for the Examination of Dairy Products* inadvertently omitted a portion of the cryoscopic method which deals with the calculation of results. The eighth edition has corrected this error.

Assume the following values on a

Bureau of Standards thermometer. For the above milk sample:

1. .514 — .425 = .089.
2. .089 × 1.015 = .0903.
3. .422 — .090 = .512 ° C.

This *true* freezing point depression of the sample is converted to percentage of added water by the use of reference tables which appear in the *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists,* or by the formula:

$$W=\frac{100\ (T-T^1)}{T}=\frac{100\ (-0.550-(-0.512))}{-0.550}=6.91\%\ \text{of added water}$$

W = % of added water

T = average freezing point of normal milk (—0.550)

T^1 = true freezing point of given sample

Normal cows' milk never gives a freezing point depression appreciably lower than 0.53 ° C. (or a freezing point higher than —0.53 ° C.); a freezing point depression lower than 0.53 ° C. (or a freezing point higher than —0.53 ° C., i.e., —0.51 ° C.) indicates a watered milk.

Presence of acidity or formaldehyde causes an increase in the depression. It was found (2) that for each 0.01 per cent increase in acidity the magnitude of the increase of the freezing point depression is approximately 0.003 ° C. In a recent report (3) it was stated that for every 100 p.p.m. of formaldehyde present, 0.009 ° C. should be subtracted from the observed depression. This increase in depression due to formaldehyde is unaltered by storage for seven days at 48 °—50 ° F. and 70° — 72 ° F.

Suggestions

1. Examine thermometer for any mercury that may adhere to glass at the top of the stem. Dislodge, if necessary, with the rubber mallet.
2. Discard any results in marked disagreement with the average.
3. Investigate erratic results by recalibration of test thermometer and by repeating the test on the sample.
4. To be assured of reliable results, determine true zero position of recently boiled distilled water and the depression produced by a standard sucrose solution each time the apparatus is used if there are long intervals between use.
5. Keep the standard (test) thermometer in an upright position.
6. Use fresh, sweet, unpreserved whole milk; those samples which show an acidity above 0.18 percent expressed as lactic acid and those with preservatives added should not be analyzed, unless acidity and concentration of formaldehyde can be determined.*
7. Calculate results with care.
8. In order to use tables for determining percentage of added water, the true (corrected) freezing point depression must be calculated first.
9. Prepare samples of milk with known amounts of water added (by volume) and determine the freezing points to check accuracy of the cryoscope.

The detailed procedure should be followed to the letter as the rate of cooling and the degree of supercooling are of extreme importance to secure accurate and reproducible results. The following precautions are given as reminders:

1. The temperature of the cooling bath (ether) should not be more than 3 ° C. below the freezing point of the milk.
2. "Maintain the temperature of the cooling bath at —2.5 ° C. and continue the manipulation of the stirrer until a supercooling of the sample of 1.2 ° C. is observed'.

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* *The Analyst,* Vol. 68, June, 1943, gives a method for determining formaldehyde in milk samples.

3. The stirring should not be too rapid and should be uniform; a rate of one full stroke per 2–3 seconds usually suffices.

4. The thermometer should be tapped with the rubber mallet repeatedly before taking a reading.

5. The size of the sample should be between 30–35 ml. of milk.

CONCLUSION

An essential adjunct to the control of the adulteration of public milk supplies is a reliable technique for detecting added water. The method should be easy to understand, to perform, and to interpret. The cryoscopic method is both reliable and practical for routine use. The practice of watering milk is increasing during our present emergency. The frequent and intelligent use of an official quantitative method will help toward halting this adulteration.

REFERENCES

1. Hortvet, Julius. The Cryoscopy of Milk. *J. Industrial and Engineering Chemistry*, **13**, March (1921).

2. Bailey, E. M. *J. Assoc. Official Agricultural Chemists*, **6**, 2754 (1923).

3. MacDonald, F. J. *The Analyst*, **68** (June, 1943).

Cheese Regulation

STATE OF CALIFORNIA

DEPARTMENT OF AGRICULTURE

A. A. BROCK, Director

Sacramento

June 16, 1944.

CIRCULAR LETTER

TO: Cheese Manufacturers

Subject: New Legislation on Cheese

Assembly Bill No. 45 passed during the 55th (4th Extraordinary) Session of our State Legislature was signed by Governor Warren and as it carried an urgency clause, it is now a law. It adds section 540 and amends section 547 of the Agricultural Code.

Briefly, the measure provides that all cheese sold in California to the retail trade shall be pasteurized or manufactured from cream, milk, or skim milk which has been pasteurized, except cheese which has been allowed to ripen or cure for a minimum period of sixty days from date of manufacture.

The measure further provides that all cheese, except processed or emulsified cheese, must be labeled at the factory where manufactured to indicate: the variety, that is, whether Cheddar, Monterey, Colby, etc.; the grade, whether whole milk, part skim, or skim; the factory number; State of origin; and date upon which the cheese was manufactured.

Cheese manufactured in any State where factory numbers are not assigned must be labeled with the name and address of the plant where manufactured.

Cheese manufacteurers are requested to give immediate compliance to this measure with respect to proper labeling and dating of the product and it is suggested that all cheese made from pasteurized products be labeled to indicate that it was made from pasteurized milk.

Your cooperation in observing the provisions of this law will be appreciated.

Very truly yours,

O. A. GHIGGOILE,
Chief, Bureau of Dairy Service,
Division of Animal Industry.