Minnesota

Dairy Products Processor

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Minnesota will soon be engaged in a testing program for mastitis and other abnormal milk conditions. The recommended test is the catalase test. To assure uniformity of testing, some characteristics of catalase and testing procedure should be considered.

SOURCE AND CHARACTERISTICS OF CATALASE

Catalases are enzymes. Enzymes hasten chemical reactions. Catalases promote the breakdown of hydrogen peroxide to water and oxygen.

Catalases are widely distributed in man. They have been isolated in crystalline form from liver, red blood cells, and kidneys. Milk catalase has not been isolated or purified.

Some bacteria found in milk contain catalase. It has been estimated that one-third of the catalase activity in "normal" milk is due to bacterial catalases. About 80 percent of the catalase activity in "abnormal" milk is due to catalase of white blood cells.

Catalase content of normal milk varies to some extent between individual cows. Feed that the cow receives also causes some variation in milk catalase content. For this reason "normal" catalase levels are usually expressed as a range (5 to 20 percent oxygen release by the standard catalase test procedure).

Optimum reaction conditions have not been defined too precisely. Generally though, it is known that catalase activity occurs over a wide range in pH. Usually activity is measured at the pH of milk, unbuffered.

Optimum temperature of catalase activity is difficult to determine. Hydrogen peroxide can destroy catalase. At high temperatures this destructive influence becomes greater. Therefore, the temperature of peak activity will be lower as higher concentrations of hydrogen peroxide are used. The amount required in the recommended procedure is one ml. of 3-percent hydrogen peroxide. This 3-percent concentration should be maintained within ± 0.2 percent (range: 2.8 to 3.2 percent). Purchase fresh stock solutions.

One individual who is very active in this area of concern says: "Within reasonable limits of temperature, results of catalase tests are similar. Certainly between 65° F. and 75° F. there is no practical difference."

Information regarding heat destruction of catalase is sketchy. Although precise temperature-time relationships of inactivation are unknown, we do know that low-temperature pasteurization causes marked catalase destruction. Only unheated milk should be tested.

RESEARCH INFORMATION ON CATALASE

A few important facts regarding catalase have been uncovered. Briefly these are:

1. Catalase activity is greater than normal in subclinical mastitis (low-grade mastitis)
2. Level of milk yield does not influence catalase activity.
3. Catalase level of colostrum milk is considerably higher than normal milk even 1 week after calving.
4. Oestrus may increase catalase activity.
5. One group of investigators states that the catalase test is a slightly better indicator of mastitis on herd milk than the California Mastitis Test (CMT). The CMT proved more satisfactory for quarter milk samples.

INFLUENCE OF PRESERVATIVES

The influence of at least two milk preservatives on CMT, Whiteside, and Catalase Test has been studied. Addition of 0.05 ml. of formalin to 10 ml. of milk greatly retarded catalase activity at oxygen levels of 21 to 170 percent. CMT and Whiteside Tests were found to decrease in positiveness, but to a lesser degree than catalase.

Potassium dichromate, another preservative, has been found to greatly reduce or eliminate reactions of all three tests.

This editor has been unable to find any results showing the influence of bichloride of mercury (corrosive sublimate) on mastitis tests. However, similar results likely would be expected. Do not make catalase determinations on preserved milk.

INFLUENCE OF STORAGE TIME

The following table shows the influences of refrigerated holding time on catalase tests:

Days refrigerated	Total no. of samples	% gas production on collection day			% gas production after refrigeration of samples		
		0-25	26-50	50 +	0-25	26-50	50 +
1	138	58	12	68	49	16	73
2	51	38	10	3	29	17	5
3	102	79	17	6	42	45	15
4	67	52	11	4	27	24	16
5	66	54	8	4	8	32	26
6	32	26	5	1	1	12	19

Using 1 day's storage above as an example, it was found that 58 of 138 samples were between 0 and 25 percent catalase level on collection day. Only 49 were in this range following 1 day of refrigerated storage. Twelve samples fell in the 26- to 50-percent range on collection day. This increased to 16 samples after storage for 1 day. In other words, catalase activity increased as a result of storage. This becomes more pronounced as refrigeration time lengthens up to 6 days as shown in the table. Analyze only fresh samples.

A similar study has been made on Whiteside and CMT. It was noted that storage had little influence on Whiteside test until the fifth day. Then samples tended to increase in positive reaction.

CMT is influenced by storage also. But CMT results become less positive as storage time increases.

DUPLICATE VS. SINGLE TESTS

On bulk milk some differences in catalase test results are noted when average of duplicate analyses are compared with a single test on the same sample. The following table compares results in three range levels of catalase activity:

% gas production	No. of duplicate tests averaged	Single tests compared to averages of duplicate tests	
		No. lower	No. higher
0-25	913	0	109 (11.9%)
26-50	224	90 (40.1%)	9 (4.0%)
50 +	46	7 (15.2%)	0

This table shows:

1. Of relatively normal samples (0 to 25 percent) a few more than 1 in 10 would have been classified higher by single test than averaged duplicates.

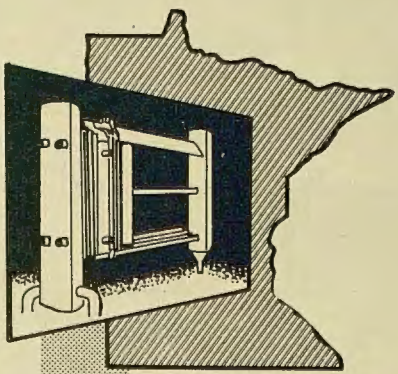
2. In the 20- to 50-percent range single tests were classified below duplicate averages 4 out of 10 times.

3. Less than one in six samples in which gas exceeded 50 percent for averaged duplicate analyses were classified below 50 percent in single tests.

Although the above variability was observed, for screening purposes it would appear that no serious drawbacks exist to use of single test determinations.

CONCLUSIONS

Precautions must be observed in making Catalase determinations if relatively uniform and meaningful results are to be obtained. Some problem areas do exist. Research likely will uncover others; but for a screening program the ultimate aim is to pinpoint problem farms so that corrective action can be taken. Experiences of those who have used tha catalase test for some time in a control effort indicate that farm problems are apparent where high catalase levels are noted. To this end, the catalase test appears acceptable. New tests have been and are being devised. Research will indicate their adaptability to a control program such as the one currently being undertaken.



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ROLAND ABRAHAM, acting director
Cooperative agricultural extension work, acts of May 8 and June 30, 1914.

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