Effects of Subclinical Mastitis on Heat Stability of Fluid Milk

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Abstract

The heat stability curves of milk taken from individual healthy and infected quarters of heifer and aged cows were examined at various stages of lactation.

It was found that at any given milking the maximum and natural heat stabilities of the milk drawn from the four quarters of healthy cows were always the same. The pooled milk from all quarters gave heat stabilities similar to those of the individual quarters. Variations in pH on different days of testing did not always cause changes in the heat stability of the milk.

Milk drawn from infected quarters generally gave much lower heat stabilities than milk drawn from healthy quarters of the same cow at the same milking. This lower heat stability was associated with a higher pH of the milk. The heat stability curves of milk from infected quarters also showed a shift to the alkaline side.

The heat stability of mixed subclinical mastitic milk and uninfected milk could not be predicted from the heat stabilities of either milk. The heat stability of late-lactation milk was high and associated with high pH.

Factors controlling the heat stability of milk proteins and their relative importance are imperfectly known and understood. Although a considerable amount of investigation into this problem was carried out from 1919 to 1935 (4, 5, 14, 21, 28-30, 32-34), little further work was undertaken for the next 20 yr.

Recently, Pyne and McHenry (18), White and Davies (35, 36), Pyne (19), Rose (22-24), and Tessier and Rose (31) have reassessed the findings of the earlier workers and presented some new concepts. For comprehensive accounts of the present knowledge of factors influencing heat stability the reader is referred to the reviews of Pyne (20) and Rose (25).

One aspect which does not appear to have been sufficiently examined is the relationship between subclinical mastitis and the heat stability of milk protein. Welch and Doan (34) stated that, despite its widespread occurrence, subclinical mastitis apparently had been disregarded as a factor in the heat coagulation of milk. White and Davies (36) stated that the milks from cows with subclinical mastitis differed considerably from the herd bulk milks in composition, but they showed no bias towards a lower or higher stability to heat.

Because of the conflicting implications of these statements, and the reported high incidence of subclinical mastitis, recent work (12, 13, 17) has disclosed that approximately 30% of milk produced comes from infected quarters (12, 13, 37); investigations were undertaken to assess the relationship of subclinical mastitis to the heat stability of milk proteins.

As Benton (4) had pointed out that each quarter of the udder represented a separate system with regard to heat stability, observations on heat stability and on the level of infection were, therefore, made on each quarter of the cows examined. Rose (22) and Tessier and Rose (31) showed that the heat stability of milk is highly sensitive to change in pH, and that milk from individual cows varies in pattern of response. Because such a pattern of response to pH gives a more complete picture of the heat stability of milk, it was adopted as the major method of observation for all the samples examined, the pH range covered being 6.4 to 7.2.

Experimental Procedures

Description of samples

(a) Source. The samples of milk were obtained from the herds of the State Research Farm, Werribee, Victoria (Red Poll and Jersey), and from two commercial herds of Jersey, Friesian, and Jersey-Friesian-cross cows. All herds were fed on irrigated perennial pasture with supplementary hay and concentrates. Cows were milked with a modified version of the quarter milking unit described by Dawson (7). Thoroughly mixed subsamples of milk, in 100-ml multiples, were taken from individual quarters or from udders of cows of varying age and stage of lactation.

Cows with four healthy quarters were sampled, as well as cows with one or more quarters with subclinical mastitic infections. In some instances the milk from individual quarters of single or several cows was pooled. All samples were taken from the complete morning milking.

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7 Present address—Department of Agriculture, Adelaide, South Australia.
Detection of subclinical mastitis. Milk from the four quarters of all cows sampled was tested to determine the presence or absence of subclinical mastitic infection. After rejecting the first two or three squirts of milk a sample was taken from each quarter and tested by the Rapid Mastitis Test (RMT). This is similar to the California Mastitis Test of Schalm and Noorlander (27). The cows were then completely milked out and a well-mixed sample of the whole milk from each quarter retested for RMT reaction. Milks were scored as negative where no slime was observed and as 1, 2, or 3 for positive reactions showing increasing sliminess. Cell counts were made on each sample to confirm the presence or absence of subclinical mastitis. In some instances bacterial estimates were made of the numbers of Staphylococci (15) and haemolytic Streptococci (9).

Preparation of samples. Separated milk was used for heat-stability tests, because the removal of the fat made it easier to detect the moment of coagulation. Previous workers had reported that removal of fat from the milk did not significantly affect its heat stability (14, 33, 35). Skimmilk was prepared by centrifuging 100-ml lots of whole milk in plastic tubes at 3,000 rpm for 10 min, chilling the tubes, and carefully removing the solidified cream plug.

Total solids. Method 15.014 of the Association of Official Agricultural Chemists (1) was used.

Milk fat. This was estimated by the Australian Standard Method no. N26 (2).

Lactose. The method used was that described by Ling (16).

Heat stability. Rose (22) has shown that the heat stability of milk is very sensitive to small changes in pH; therefore, great care was taken to ensure that all glassware was carefully washed and double-rinsed in ion-free water before use. Unless otherwise stated, the pH of the milk samples was adjusted by addition of small amounts (0.1-2.0 ml per 20 ml milk) of 0.5 M sodium dihydrogen phosphate or dipotassium hydrogen phosphate. All the 20-ml lots of milk were made up to 22 ml with ion-free water to minimize any effects of dilution on pH or heat stability (see Results).

After pH adjustment, samples were equilibrated overnight at 4 C. The heat stability test was carried out next day by transferring 2-ml aliquots into thick-walled, constricted 6 by 5/8-in Pyrex test tubes which were sealed, attemperated for 30 min at 30 C, clipped into a metal rack holding 15 ampoules, and completely immersed in a silicone oil bath equipped with a Haake* stirrer, and maintained at 140 ± 0.1 C. The ampoules were gently rocked, continuously for the first 2 min and thereafter for 10-15 sec every 2 min. The purpose of this was to heat the milk evenly and minimize the baking-on on the glass surface of the coagulating whey proteins, since such baking-on increased the difficulty of observing the point of coagulation.

Every 2 min the samples were removed from the bath, slowly tilted to allow the milk to flow, and observed for the presence of clots. In some samples very fine precipitates occurred and a magnifying glass (2.5×) was used to facilitate observation.

The time recorded for coagulation was measured from the moment of immersion and all tests were duplicated in separate runs. The difference between duplicates tested in this way was found to be not more than 2 min. The method described is similar to the test used by Miller and Sommer (17), Cole and Tarassuk (6), White and Davies (35), Rose and Tessier (26), and Belec and Jenness (3).

pH measurements. All samples were examined after overnight equilibration at 4 C. Samples were adjusted to 20 C. and the pH determined with a Jones Model R null balance potentiometer equipped with a dual electrode. Care was taken to maintain the whole system at 20 C inside a temperature-controlled box, as accuracy to ± 0.0025 pH unit was required. [Dixon (8) has shown the temperature coefficient of the pH of milk to be 0.01 pH unit per 1 C]

Results

1. Preliminary studies. These were made to establish the influence on heat stability of variations in testing technique.

(a) Storage of samples. The number of samples that could be examined in one day was limited and the effect of storage for up to three days was, therefore, examined. Samples were stored by holding at 4 C or by preservation with 0.3 ml of 10-vol hydrogen peroxide per 100 ml of milk. Aliquots were taken daily and tested for heat stability. Storage at 4 C was found not to affect the heat stability curves, but hydrogen peroxide treatment gave variable results.

(b) Dilution effect. Although Rose (22) has stated that small dilutions of milk with water had no effect on heat stability, this factor was

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* Dow Corning 200/220 Fluid Silicone.

* Haake Unitherm Thermostat and Circulator, Gebruder Haake, Berlin, West Germany.
re-examined. Using up to 3 ml of ion-free water per 20 ml of milk had no effect on heat stability, although a slight shift in pH was observed. In contrast to the procedure of Rose (22) all samples in a series had a constant dilution factor.

There was no difference in heat stability whether the milk was added to the electrolytes or vice versa, nor did the method of mixing have any effect, but overnight equilibration at 4°C was necessary to give reproducible results.

(c) Temperature of testing. Cole and Tarasuk (6) and White and Davies (36) showed that, in general, there was a straight-line logarithmic relationship for natural milks between heat-stability tests carried out at different temperatures. This relationship was investigated for milks adjusted in pH over a range of 6.4 to 7.2. The times of coagulation at the various pH levels decreased geometrically with testing temperature, i.e., the temperature coefficient (Q10°C) was found to be approximately two between 120 and 130°C and between 130 and 140°C. As sufficient differentiation in heat-stability times was observed at 140°C, and the heat stability tests were not extended, this temperature was adopted.

2. Heat-stability curves of milk from healthy cows. To establish a basis for comparison, the heat-stability curves of milk from all quarters of three heifers that remained free of infection during their first lactation were examined. [The history of infection of 27 heifers from the commencement of their first lactation was recorded, so that cows of known background, i.e., free of infection, were used (11)]. Results indicated that whereas the maximum heat stability [defined as that occurring in the range of pH 6.6-6.9, c.f. Rose (22)] of the milks from each of the four quarters of a cow were the same at any one milking, their magnitude could vary from day to day. Furthermore, there was often a variation from day to day in the pH at which the maximum heat stability of the milk occurred.

Although on any one day the pH of the unadjusted milk of all four quarters was not necessarily the same, the heat stability was. For any given quarter, the pH was not constant from day to day, but these fluctuations in pH did not always alter the heat stability of the milk. An example of one cow tested on two occasions is given in Table 1 (see also Figure 1). Heat stability curves of the pooled milk of all four quarters of each cow were similar to those exhibited by the individual quarters.

3. Effect of subclinical mastitis. It will be seen in Figure 1 that a random natural increase in pH of the milk from a healthy quarter did not affect the heat stability, but when the pH of
Comparison of the heat-stability curves of RMT-negative and -positive milks from one cow. ○ Right hind quarter 23·10-64 (negative); ● right hind quarter 26·10-64 (negative); △ left hind quarter 26·10-64 (positive); → heat stability of sample before pH adjustment.

The milk was increased by subclinical mastitis and the heat stability was drastically reduced. Subsequently, comparisons of the heat stabilities of RMT-negative and -positive milks were made on 37 pairs of quarters. The results, summarized in Table 2, show that a high proportion of the RMT-positive quarters are lower in heat stability than the corresponding negative quarter from the same cow. Compared with the heat stability curves of milk from RMT-negative quarters of cows, the milk from RMT-positive quarters usually showed a slight shift to the alkaline side.

When RMT-negative and -positive milks from the same cow were mixed, the resultant heat stability could not be predicted. The coagulation time could be the same as either milk, somewhere between, or even lower than either milk. However, where the RMT positive milk was considerably higher in pH (pH > 7.0) than the RMT negative milk, the heat stability of the mixture was closer to the former.

An investigation was undertaken to ascertain whether there was any interaction between pH and RMT reaction and its relationship to heat stability. Milks from 99 quarters from a group of 50 cows were examined. Results are presented in Figure 2. Where the milk had a high pH and a positive RMT reaction, the heat stability was low. Within the pH range 6.60-6.80 there was no relationship between pH and heat stability (the majority of these samples were RMT negative). However, the average heat stability of the RMT negative milk (26 min) was twice that of RMT positive milks (13 min).

4. Heat-stability curves of late-lactation milks. Feagan and Griffin (10) observed that in a group of heifers in their first lactation there was a sharp increase in the incidence of positive RMT reactions towards the end of lactation. Although it has just been shown that RMT positive milk had a low heat stability, previous workers (35, 36) had stated that late lactation milk tended to have longer coagulation times than normal milks. Individual quarter samples were, therefore, obtained from five cows during their last week of lactation (they were yielding between 6 and 15 lb of milk per day).

These samples were analyzed for chemical composition, bacterial population, cell count, RMT reaction, and heat stability curves.

The solids-not-fat contents were considerably higher (average, 12.0%) than for mid-lactation milk. The lactose content averaged 5.0%. In the majority of samples there was a positive RMT reaction associated with a high cell count (> 3 \times 10^6/ml), but the total bacterial count was low (<1,000/ml) and no organisms associated with subclinical mastitis were isolated. The pH of the natural (pH unadjusted) milk was high (pH 7.00 and above), but this was associated with a high heat stability (30-50 min). Figure 3 shows the progression of a heat stability curve over four days during the last week of lactation. As the natural pH increased from below to above 7.00 the heat stability markedly

**Table 2**

<table>
<thead>
<tr>
<th>Description</th>
<th>No. of RMT +ve quarters lower than -ve quarter</th>
<th>No. of RMT +ve quarters with no difference</th>
<th>No. of RMT +ve quarters higher than -ve quarter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of differences (min at 140°C)</td>
<td>24</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Average differences (min at 140°C)</td>
<td>2.34</td>
<td>--</td>
<td>2.6</td>
</tr>
<tr>
<td>Range of differences (min at 140°C)</td>
<td>15</td>
<td>--</td>
<td>5</td>
</tr>
</tbody>
</table>
MASTITIS AND HEAT STABILITY

FIG. 2. Effect of interaction of pH and RMT reaction on heat stability. Observations made on individual quarters. O RMT-negative; • RMT-positive.

increased. In contrast to mid-lactation milk the heat-stability curves do not show the characteristic maximum in the pH range 6.55 to 6.75.

Discussion

The studies reported in this paper support the statements regarding the effects on heat stability of milk of the source of the sample (5) and of the presence or absence of subclinical mastitis (34).

The present studies have shown that milk from quarters infected with subclinical mastitis is markedly different in heat stability from the milk of healthy quarters of the same cow. This effect appears to be over and above the variation in natural heat stability of milk from healthy cows. Therefore, by using only animals known never to have been infected with subclinical mastitis can the original heat-stability patterns for individual quarters of a cow be established.

Variations found in the heat stability of natural milk from individual quarters on different occasions of sampling agree with results obtained for the total milk from the udder by Rose (22). In addition, the heat stabilities of the natural milks from the four quarters of one cow at any milking were the same. This is at variance with the observations of Benton (4).

Although our observations agree with those of Rose (22), that small alterations made in pH by addition of electrolytes can alter the heat stability of milk, it was found that when similar small changes in pH occurred naturally in healthy quarters there was not always a corresponding change in the heat stability of the milk.

In contrast to the findings of Benton (4) and Rose (22), that late lactation milk has a low heat stability, our observations agree with those of White and Davies (36), that such milk is more heat-stable. In the present study high heat stability times in late lactation milk were associated with natural pH values greater than 7.00.

All the heat-coagulation times at 140°C observed in these studies were distinctly higher than those reported elsewhere in the literature.

The presence of subclinical mastitis in a quarter can introduce a tremendously complicating factor into any investigation of the heat stability of milk based on the pooled milk of all four quarters. Pooling RMT-negative and -positive milks can give a variable heat stability for the mixture. This could account for
some of the variations in heat stability observed by Holm et al. (14) and Rose (22) for individual cows over a period of time. This factor, together with the wide range of natural heat stabilities observed for milk from quarters of healthy cows (see Figure 2), could explain why White and Davies (36), using separate animals, obtained no significant difference between the average heat stability of mid-lactation milk of healthy cows and milk from cows with subclinical mastitis.

The wide variation in heat-stability time recorded within the pH range 6.60 to 6.80 confirms conclusions of Benton and Albery (5), and of most later workers in this field, that the heat stability of natural milk is not related to its pH within this range. However, for milk from infected quarters there is a relationship between heat stability and pH above pH 6.80.

Results reported here indicate that any future work on the heat stability of milk will have to take into account many factors, the most important being complete control over the source of samples and recognizing the complicating effects of natural variations and those caused by subclinical mastitis.

The authors believe that subclinical mastitis adds yet another factor to those known to influence the heat stability of milk.

References


