UTILIZATION OF NATURAL LACTOPEROXIDASE SYSTEM TO EXTEND KEEPING QUALITY OF RAW MILK

B. G. Harnuly* and A. Hamid**

ABSTRACT: Morning samples of bovine as well as buffalo milk were stabilized by activation of the naturally occurring lactoperoxidase (LP) system. In each case, the keeping quality of a stabilized sample was compared with that of a control. The 10 minute resazurin test was the main method used to analyze milk quality. The results show that good quality morning milk, stabilized shortly after milking and stored at about 30°C during the day, will last until the late afternoon. This method of milk quality preservation may be used to encourage dairy farming by making possible the collection of more milk of high quality which in turn is a pre-requisite for the increased manufacture of high quality dairy products.

Key Words: Milk; Keeping Quality; Enzyme.

INTRODUCTION

The lactoperoxidase system consists of the enzyme lactoperoxidase (LP), which catalyzes the oxidation of thiocyanate (SCN⁻) by hydrogen peroxide (H₂O₂) to compounds showing specific anti-bacterial activity.

The LP system seems to have a wide distribution in nature. Extensive work on its occurrence and function in human and animal milk has been carried out (Reiter, 1978; Bjorck, 1980; Korhonen, 1980; Reiter and Harnuly, 1982). As other examples of the natural occurrence, the LP system has been reported to be active in human saliva (Thomas et al., 1981) and in the calf abomasum (Reiter et al., 1980).

To activate the LP system in milk, the natural concentration of SCN⁻ (normally 3–5 ppm) should be increased to about 15 ppm, a concentration which is still much lower than reported values for human body fluids such as saliva and gastric juice (Ruddell et al., 1977). Moreover, for optimum activity, H₂O₂ should be present at equimolar concentration with SCN⁻ (Bjorck, 1978). Since any H₂O₂ generated in vivo is rapidly dissociated into water and oxygen when the milk has left the udder, 8 to 10 ppm of H₂O₂ has to be added. This concentration is much lower than those (upto 800 ppm) considered by FAO/WHO for the preservation of milk (Luck, 1962).

Early research on the anti-microbial activity of the LP system revealed an inhibitory effect on Gram-positive bacteria such as streptococci (Reiter et al., 1964). Later, a strong effect against Gram-negative psychrotrophs was demonstrated (Bjorck et al., 1975; Reiter et al., 1976) suggesting a possibility to reduce the frequency of collecting refrigerated farm milk. More recently, experiments in Kenya (Bjorck et al., 1979) and Sri Lanka (Harnuly and Kandasamy, 1982) showed that the keeping quality of raw uncooled milk can also be substantially extended by activation of the LP system. The present work was undertaken to study the potential advantages of using this method of milk quality preservation under Pakistani conditions.

MATERIALS AND METHODS

Sampling

Morning milk from four farms and five Milk Collection Centres (M.C.C.) in the Sahiwal district of Pakistan was studied.
Small samples of milk from milking buckets or churns were collected in 100 ml polyethylene bottles. A stabilized sample was always compared to a control, stored at the same temperature. When stored at two different temperatures, four samples were consequently drawn from each bucket or churn.

While sampling information about the type of animals, the time of milking, and the ambient temperature was recorded.

Activation of LP System

Samples taken at farms were generally stabilized in the laboratory of the dairy plant at Renala, Sahiwal, whereas M.C.C. samples were stabilized at the centres. Activation of the LP system (stabilization) was carried out as follows. First, 1 ml of a 20 mM aqueous solution of NaSCN (Mallinckrodt, U.S.A.) was added to the 100 ml sample of milk. The concentration of thiocyanate was thus increased by 11.6 ppm. Then, 1 ml of a 25 mM aqueous solution of H₂O₂ was added and rapidly mixed with the milk to give an initial concentration of 8.5 ppm. The 25 mM solution of H₂O₂ was prepared shortly before each experiment by adding 0.28 ml of a 30 percent H₂O₂ solution (Merck, W. Germany) to 100 ml of distilled water.

Storage Temperature

Most of the samples were stored in the above laboratory at ambient temperature (usually about 30°C). Some samples were, however, stored outdoors to study the influence of higher storage temperatures. At around noon, outdoor sample bottles became exposed to sunshine resulting in a temperature increase of about 40°C and 50°C. To protect the milk from the UV radiation of the sun, these bottles were wrapped in non-transparent tape.

Analyses

Resazuring readings were determined by mixing 1 ml of a 0.005 percent freshly prepared aqueous solution of resazurin (BDH, England) with 10 ml of milk and incubating at 37°C for 10 mins. Milk samples were recorded as rejected if the resazurin reading was lower than 5.

Hygicult® dip slides (Orion Diagnostica, Finland) were used in the field to estimate initial viable counts of bacteria. Curdling and clot-on-boiling were also studied.

RESULTS AND DISCUSSION

Good Quality Milk

The results presented in Table 1 and the figure show that if the bacteriological quality of the milk is good when it is stabilized, a considerable extension of its keeping quality can be achieved. Milk samples stabilized at about 0700 h and kept at 30—32°C during the day remained acceptable for 10½ to more than 12 hours. Table 1 also shows that activation of the LP system in buffalo milk apparently results in an extension of its keeping quality as that in bovine milk.

Effect of Outdoor Temperature

Even at higher storage temperatures, the rate of spoilage of milk can be greatly reduced by activation of the LP system. Figure shows that stabilized milk samples stored at about 30°C and those stored at a maximum temperature of about 45°C have approximately the same keeping quality. Further experiments revealed that if the peak temperature of the outdoor samples is increased to 49°C, some of these samples actually spoiled later than those stored in the laboratory at about 30°C. Contrary to this, a maximum temperature of 41°C consistently resulted in slightly reduced keeping quality.

All outdoor controls spoiled at about the same time as the corresponding samples stored in the laboratory.

Influence of Initial Bacteriological Quality

There is an inverse relationship between
Table 1. Effect of activation of the LP system on the keeping quality (10 mins resazurin test) of buffaloes and bovine milk from the New Normandi Farm, Okara

<table>
<thead>
<tr>
<th>Type of animal</th>
<th>Time of milking (h)</th>
<th>Hygicult® reading (10^3 x bacteria/ml)</th>
<th>Time when spoilage observed to start (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffalo</td>
<td>0640</td>
<td>5</td>
<td>1440</td>
</tr>
<tr>
<td>Buffalo</td>
<td>0650</td>
<td>8</td>
<td>1440</td>
</tr>
<tr>
<td>Buffalo</td>
<td>0710</td>
<td>30</td>
<td>1545</td>
</tr>
<tr>
<td>Cow</td>
<td>0655</td>
<td>30</td>
<td>1545</td>
</tr>
<tr>
<td>Cow</td>
<td>0645</td>
<td>50</td>
<td>&lt;1440**</td>
</tr>
</tbody>
</table>

* All stabilized sample passed the test at 1945 h
** Before 1440 h

Note: Time of stabilization, 0740 h (ambient temperature 25°C), during the day, samples were kept at 31–32°C.

Stabilization of raw milk by activation of the LP system (Solid lines: Samples stored at around 30°C, Broken lines: Samples stored at higher temperatures, Milk temperature: 43°C at 1400 h, 46°C at 1500 h and 45°C at 1630 h).

bacteriological quality of the milk at the time of stabilization and the time until spoilage (Table 2).

A considerable variation in the initial bacteriological quality of the samples was observed. Table 3 shows the percentages of farm and M.C.C. milk samples with initial bacterial counts below or equal to 10^5/ml, from more than 10^3/ml to 10^4/ml, etc. The mean value of the farm milk samples falls in the range of 10^4/ml and 10^5/ml whereas most of the M.C.C. milk samples have counts well above 10^6/ml.

Observations on Methods of Analysis

Duplicate samples with Hygicult® dip slides always gave very similar results. A close relationship between these readings and the keeping quality was generally observed.

On average, spoilage according to the clot-on-boiling test was recorded more than two hours later than spoilage according to the 10 mins resazurin test.

The results show that good quality milk (bacterial count below 10^5/ml), stabilized in the morning and stored at around 30°C will last until the late afternoon or, in many cases, till evening. This is well in accordance with earlier experience from Kenya (Bjorck et al., 1979) and Sri Lanka (Harnulv and Kandasamy, 1982). The results at 30°C shown in the figure are almost identical to those obtained at Narahenpita M.C.C. in Sri Lanka. Another similarity is the finding that buffalo milk is as
Table 2. Relationship between initial bacteriological quality and keeping quality (10 mins resazurin test) of LP stabilized milk samples

<table>
<thead>
<tr>
<th>Time of stabilization (h)</th>
<th>Average Hygicult\textsuperscript{®} reading (x 10\textsuperscript{8} bact/ml)</th>
<th>Time when spoilage observed to start</th>
</tr>
</thead>
<tbody>
<tr>
<td>0740</td>
<td>25</td>
<td>&gt; 19.45*</td>
</tr>
<tr>
<td>1045</td>
<td>640</td>
<td>17.00</td>
</tr>
<tr>
<td>1045</td>
<td>8100</td>
<td>14.40</td>
</tr>
</tbody>
</table>

* All stabilized samples still good at this time.

Note: Time of milking 0640–0800 h. Hygicult\textsuperscript{®} samples were taken just prior to stabilization; during the day, samples were kept at 30 to 32\textdegree C.

Table 3. Bacteriological quality of farm and M.C.C. milk samples

<table>
<thead>
<tr>
<th>Bacteria/ml of milk</th>
<th>Percentage distribution of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Farm samples</td>
</tr>
<tr>
<td>(&lt; 10^3)</td>
<td>6</td>
</tr>
<tr>
<td>(&gt; 10^3) to (&lt; 10^4)</td>
<td>12</td>
</tr>
<tr>
<td>(&gt; 10^4) to (&lt; 10^5)</td>
<td>64</td>
</tr>
<tr>
<td>(&gt; 10^5) to (&lt; 10^6)</td>
<td>12</td>
</tr>
<tr>
<td>(&gt; 10^6) to (&lt; 10^7)</td>
<td>6</td>
</tr>
</tbody>
</table>

Easily stabilized by activation of its LP system as bovine milk. This may be of importance to a country like Pakistan, accounting for almost one-fourth of the world production of buffalo milk (FAO, 1982).

The results obtained with the milk samples stored outdoors at temperatures up-to 45–50\textdegree C are encouraging since they show an extension of the keeping quality similar to that in samples stored at about 30\textdegree C. The possible explanation that this is a result of the optimum temperature for bacterial growth and quicker multiplication does not seem to apply because high temperature controls spoiled after about the same time as those stored at about 30\textdegree C. There might be a change in the bacterial population towards more LP sensitive organisms as the temperature is increased. More research will, however, be needed to establish in greater detail the reason(s) for this surprisingly good keeping quality at temperatures in the range 45–50\textdegree C.

Table 2 illustrates that the better the quality of the milk at the time of stabilization the more extended is its quality keeping time. These results further illustrate the basic principle that stabilization of milk by activation of its LP system is a method to retain good quality in milk but not a means of concealing or improving poor quality milk.

Much of the milk arriving at the collection centres at 1000–1200 h, however, showed a poor or even very poor bacteriological quality (Table 3). The fact that most farm milk samples were quite good, demonstrates the need for more thorough cleaning of milk vessels and for improved milk collection, perhaps by collecting the milk more rapidly and/or preserving the milk quality (cooling,
heating + cooling, LP activation) at an earlier stage.

The observation that the clot-on-boiling test shows spoilage a few hours later than the 10 mins resazurin test also compares well with earlier findings in Sri Lanka (Harnulv and Kandasamy, 1982). This illustrates the importance of stating which test has been used as a criterion of spoilage.

It may be concluded that this method of milk quality preservation may be used to encourage dairy farming by making possible the collection of more milk of high quality which in turn is a pre-requisite for the increased manufacture of high quality dairy products.

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LITERATURE CITED