POTENTIALIZATION OF THE LACTOPEROXIDASE SYSTEM FOR PRESERVATION OF RAW MILK IN THE TROPICS[1]

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SUMMARY

The antimicrobial ability of the lactoperoxidase system was increased by the addition of larger amounts of the thiocyanate and hydrogen peroxide at levels above those suggested by other authors. Results of laboratory and field trials revealed that the potentialized system was able to preserve poor-quality raw milk for longer periods of time, at "tropical" temperatures, than when used as recommended previously. It was possible to preserve some milks at 20°C for more than one day, without diminishing their overall quality. At 36°C, the milks did not show acidity development for about 10 hours. Tests conducted under real collection and transportation conditions validated these findings. It was therefore proved that the system can be used practically and that its bactericidal/bacteriostatic effect on the spoilage flora of milk can be increased in order to overcome the particularly adverse conditions of milk handling in the tropics.

INTRODUCTION

Preservation of raw milk in some tropical regions poses a number of difficulties, mainly in its handling and transportation process, from the farms to the processing or consumption sites. Those transportation periods vary from 2 to 8 hours, or even longer. Milk producers of the tropics in developing countries usually do not count with an adequate infrastructure for the appropriate handling of raw milk. This situation forces producers to find methods for raw milk preservation, the addition

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of products that represent a potential public health hazard, for example, formaldehyde, antibiotics and others (1). Due to the fact that in the short run it is not possible to foresee an immediate technification of the milk handling procedures, it becomes necessary to look for alternative preservation technologies. These technologies should be capable of protecting raw milk from spoilage for periods long enough to assure its safe handling from the farm to the processing plant. They must be simple to use, and not pose any kind of hazard for the consumer.

The basics of the lactoperoxidase/thiocyanate/hydrogen peroxide (LP system) have been covered during the past years (2-10). Through previous research, it has been established that in order to activate the system, the natural content of thiocyanate (SCN^-) in milk should be raised to 0.25 mM/lt, with the subsequent addition of the equimolar amount of hydrogen peroxide (H_2O_2) (5, 11). The enzyme will then carry one oxygen molecule from the H_2O_2 to the SCN^-, which is converted mostly to $OSCN^-$, a bacterial inhibitor that is more effective against gram-negative and pathogens than the use of lactic bacteria.

The purpose of the work herein presented was to prove that the antimicrobial power of the LP system can be increased by raising the SCN^- and H_2O_2 levels in equimolar proportions, since the enzyme is not limiting.

MATERIAL AND METHODS

Our research work was carried out in two stages, first under temperature-controlled laboratory facilities, and second in field trials, working in collaboration with a 20,000 lt/day milk pasteurization plant.

For the first study (12), fresh raw milk was obtained from "La Posta" Animal Research Center located 18 km from Veracruz, with mechanical milking and a mixed Holstein-Brown Swiss herd. Fresh milk was taken to the laboratory in clean containers, at ambient temperature, within 60 minutes following milking. At the laboratory, samples were analyzed for: titratable acidity (T.A.), pH and standard plate count (SPC), according to the Standard Methods for the Examination of Dairy Products (13). The level of SCN^- naturally present in each milk was also determined as follows: to 10 ml aliquots of the milk, 10 ml of 20o/o trichloroacetic acid were added; the mixture was then centrifuged at 3,000 rpm for 10 minutes, and the supernatant filtered through Whatman No. 2 filter paper; 5 ml of the Sörbo reagent (100 g of $Fe(NO_3)_2.9H_2O$ dissolved in 65o/o HNO_3) were added to 1 ml of the filtrate, and taken to 1 lt (with distilled water); the mixture was allowed to stand for 4 min and its absorbance read at 460 nm in a Carl Zeiss PM 2K spectrophotometer. Once the SCN^- level of the milk was known, the LP system was activated in duplicate samples at the 1X, 2X, 3X, 4X and 5X potencies, defining potency 1X as the concentration of SCN^- and H_2O_2 of 0.25 mM/lt, 2X to 0.50 mM/lt, and so on. The SCN^- and H_2O_2 levels were fixed by direct addition from stock solution of NaSCN, and prepared fresh daily from 30o/o H_2O_2 (both Baker, A. R.). The remaining H_2O_2 was monitored with $TiCl_4$, by the method of Ferrier, Olson and Richardson (14). The LP-treated milks, along with their respective controls were stored in laboratory incubators

set at 20, 24, 28, 32 or 36 ± 0.5°C. Their deterioration was followed at regular intervals until spoilage was evident.

The second study (15) was carried out under actual conditions of milk collection and transportation used by a milk pasteurization plant located in the county of Veracruz. Each of the four collection routes (A, B, C and D) was studied in terms of time required for the milks to reach the plant, and temperatures at which the milks arrived. At the plant, at least 20 samples of each route were analyzed for their SCN^- content to determine an average for each route. Later, additions of SCN^- and H_2O_2 were calculated and performed at the collection points, using stock solutions of the reagents, prepared at the plant laboratory and carried cold. The LP system was activated first in 500 ml samples at potencies 1X to 4X. Two controls were used: one refrigerated sample with nothing added, and one at ambient temperature wherein the chemical currently used by the plant as preservative was added (ClO_2). In a second set of trials, the system was activated in 40 lt cans at potency 3X. In both sets of runs the milks were analyzed upon their arrival for SPC and T.A.; their temperatures and transportation times were also recorded.

RESULTS AND DISCUSSION

The behavior of milks stored at 28°C, which may be considered a temperature typical of those found in the tropics, is presented in Figure 1. The ordinate was transormed to increments in T.A., as compared to the initial values, since this was variable, ranging from 0.14 to 0.18°/o. Each point is the average of three runs. As observed, for some treatments, shortly after the activation, the T. A. decreased below the initial value. This has been a common finding in previous works of our team, but has not been reported in the literature. Although the exact origin of this acidity loss was not determined, it is supposed to be due to the formation of OH^- radicals or to the neutralization of hydrogen ions by reaction with a system product or by-product.

Observing the development of T. A. of the milks activated with the system at different potencies, it is possible to notice an inversely proportional relationship between potency and rate of acidity development. Potency 1X —which is the one recommended by researchers working in other countries as Sri Lanka (16), Poland (17, 18), Kenya (11, 19), and also in Mexico (20)— does have some preservation ability, but it is much smaller than the other potencies and would not seem likely to solve the problem of milk transportation for long periods of time at high temperatures. The other potencies show that the quality of the milks could be extended up to 14 hours. These results could appear very sound, but considering the fact that the experiments were made with "good" quality milk, with a standard plate count (SPC) of about 10^5 cfu/ml, preservation times would surely decrease with poor-quality milks, as those usually handled in the tropic.

The results of all the runs at the temperatures and potencies stated above are condensed in Table 1, in terms of preservation time, defined as the time in hours required for the T. A. to reach 0.01°/o above its initial value. Preservation times decreased with temperature, and were minimum

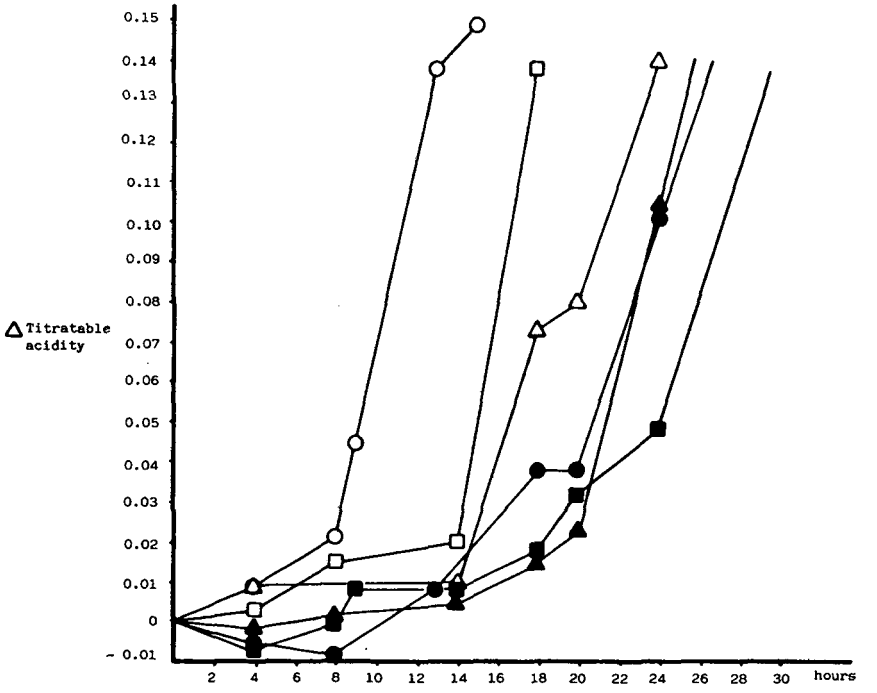

FIGURE 1

Development of Titratable Acidity in milks stored at 28°C. ○ Control □ Milk treated with the LP system at potency 1X, △ potency 2X, ● potency 3X, ▲ potency 4X, ■ potency 5X

at 32°C which apparently, is the optimum growth temperature for the natural flora of milk. Björck (11) reported preservation times for potency 1X of 7-8 hours at 30°C, and 15-16 hours at 20°C. It may be considered that, although we are comparing two different experiments made in two different countries and milks, our preservation times achieved with potencies above 1X are much higher than hose reported previously. Based on our data, we can further suggest that milks similar to those used in our tests, stored at an average temperature of 20°C, common in higher lands with temperate climate, may keep its original T. A. for over one day, allowing every-other-day pick up. At 28°C milks can be kept without change for over 12 hours, and for about 10 hr at 36°C, the last two temperatures being common in the tropics. Nevertheless, those times should allow the producer to transport his milk to the processing plant, where it is rapidly cooled. A strong limitation arises when the storage or transport temperature is close to 32°C, since the system is overpowered in

TABLE 1

EFFECT OF ACTIVATION OF THE LP SYSTEM POTENTIALIZED IN RAW MILKS STORED AT DIFFERENT TEMPERATURES (PRESERVATION TIMES IN HOURS REQUIRED TO OBSERVE A CHANGE IN 0.01°/o OF THEIR INITIAL T.A.*)

Sample	20°C	24°C	28°C	32°C	36°C
Control	8	6	4	4	4
1X	19	10	6	3	5
2X	27	13	14	5	—
3X	26	—	13	3	10
4X	26	23	16	6	—
5X	22	—	13	9	11

* Dashes indicate runs not performed.

less time by microbial growth. In such a situation, a viable alternative would be to periodically reactivate the system by the addition of H_2O_2 only, since the level of SCN^- is only slightly reduced.

Based on the encouraging results derived from the laboratory study, it was decided to take the system to work in the field. From initial observations, it had been noticed that the milks were of a very poor microbiological quality, with SPC in the range of 10^6-10^8 cfu/ml. Other finding was that the SCN^- content of milks from each route was variable having detected the following values:

Route	SCN^- (mM/lt)
A	0.19 ± 0.072
B	0.11 ± 0.034
C	0.12 ± 0.038
D	0.15 ± 0.063

Depending on the SCN^- content, the milk from each route was treated differently, using its own average SCN^- concentration as the basis for calculation of the addition of exogenous SCN^-, in order to fix its levels at those required for the different potencies. Among a number of experimental runs on each route, Figure 2 shows the T. A. and SPC of the different milks in experiments with 500 ml. If the original quality of the milk is considered as the one represented by the refrigerated sample, we can see that potencies 1X, 2X and 4X were unable to retard acid production and bacterial proliferation. It seems clear that there is a kind of additive effect between potency and bacteriostasis, being potency 3X apparently the most adequate in this run, and also in most of the others. Again, the high initial bacterial counts are noticeable, which is the hardest difficulty to overcome with any preservation system. On the other hand, the chemical of current use by the plant did not compare with any of the potencies

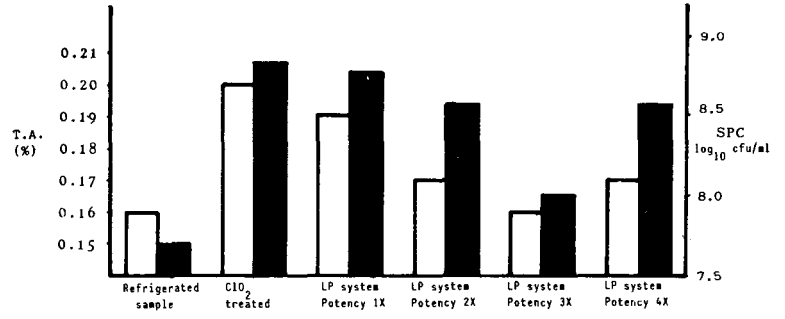

Figure 2. Titratable acidity (void bars) and Standard Plate Count (solid bars) of raw milk samples in 500 ml bottles. The milks were collected in route C, with a transportation time of 6 hours and temperature at arrival of 32°C.

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Titratable acidity (void bards) and Standard Plate Count (solid bars) of raw milk samples in 500 ml bottles. The milks were collected in route C, with a transportation time of 6 hours and temperature at arrival of 32°C

of the LP system, being the milk treated with ClO_2 within the range of T. A. observed in every day operation during the summer. In most of the other runs, the pattern was similar, concluding, therefore, that the use of the LP system offers a better choice.

With the system tested in all routes under different times and temperatures, it was found that the potency 3X appeared to be the most adequate for our conditions. A series of runs was made using this potency in all routes, but in 40 lt cans, that is, the unit for regular handling of the raw milk by the plant. An example of the 40 lt runs is presented in Figure 3. Again, the milk treated with the system was similar in T. A. and SPC to the refrigerated sample, and better than the rest of the milk treated with ClO_2. There were other runs that involved longer transportation times and higher temperatures; in all of them, except when there was mishandling, the LP system proved its effectiveness, yielding milks with characteristics that still allowed their processing into pasteurized milk.

One of the important points of the work herein reported is the fact that, providing that the plant's chemist prepared the reagent solutions, the truck driver was able to activate the system at the collection points, simply by adding in order the contents of two tubes, which were carried cold in the truck's cabin. This proposes that, with adequate technical supervision, there should not be any need for specialized training of the milkhandlers.

With reference to the possible health risks for using the system, the only concern could arise from the SCN^-, which is known as a goitrogenic agent. However, an excellent review by Reiter and Härnulv (21) depicts the most important studies made so far on this subject. It is possible to say that even using potency 3X –which represents a level of about

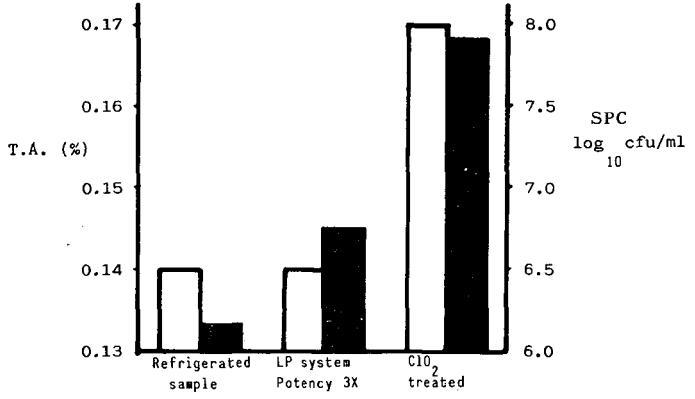

FIGURE 3

Titratable acidity (void bars) and Standard Plate Count (solid bars) of raw milk in 40 lt cans. The milks were collected in route B with a transportation time of 6 hours and temperature at arrival of 33°C

42 ppm of SCN^-, the system is not expected to indirectly pose any risk for the consumer. The H_2O_2 does not present any health hazard because it fades out a few hours after its addition, since it is consumed as substrate by the system, yielding H_2O. Finally, the inhibitor formed by the reaction, the $OSCN^-$ among others, is not stable to the pasteurization treatment, so there is no inhibitor remaining; in the event there were any, the same review by Reiter and Härnulv states that it will not affect adversely human cells or organs. Furthermore, it has been suggested by some researchers that the LP system exists in the mouth and may play a role against the microorganisms responsible for oral cavities (22).

Finally, it must be strongly stressed that no chemical or biochemical method of milk preservation should be used to substitute or mask poor hygienic practices of milk handling, and that milks with original high bacterial counts cannot yield good-quality manufactured products. We propose a system that may contribute to relieve the hot problem of raw milk preservation in warm regions of developing countries. In the medium to long term, these regions should be able to adopt refrigerated transportation and storage for their product, along with better sanitation practices for its handling.

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RESUMEN

POTENCIALIZACION DEL SISTEMA LACTOPEROXIDASA PARA LA PRESERVACION DE LECHE CRUDA EN LOS TROPICOS

Se aumentó la capacidad antimicrobiana del sistema lactoperoxidasa, mediante la adición de tiocianato y peróxido de hidrógeno en cantidades mayores a las sugeridas por otros autores. Los resultados de laboratorio y las pruebas de campo revelaron que el sistema potencializado pudo preservar leches de baja calidad microbiológica, a temperaturas "tropicales" por períodos más largos que al usarlo como se recomienda en la literatura. Se pudo conservar leches a 20°C por más de un día, sin menoscabo de su calidad general. A 36°C, las leches no acusaron desarrollo de acidez durante el término de 10 horas. Las pruebas realizadas en condiciones reales de recolección y transporte validaron los resultados de laboratorio.

Se logró así probar que el sistema lactoperoxidasa es viable de uso en la práctica, y que su poder bactericida/bacteriostático sobre la flora deterioradora de la leche puede aumentarse a fin de superar las condiciones especialmente adversas que involucra el manejo de la leche en los trópicos.

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