Korean J. Appl. Microbiol. Bioeng.
Vol. 5, No. 3, 113~118 (1977).

Development of Temporary Preservation Method for Small Scale Dairy Farm Milk by H_2O_2 Catalase Treatment

(Part 1) Bactericidal Effect of Hydrogen Peroxide and Its Stability in Milk

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(Received July 5, 1977)

H_2O_2-Catalase 처리에 의한 소규모 목장우유의 일시적 보존법의 개발

(제 1 보) 우유에 있어서 과산화수소의 살균효과 및 안정성

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(1977년 7월 5일 수리)

Abstract

Into the precontaminated farm milk hydrogen peroxide (H_2O_2) was added at the concentrations ranging from 0.01% to 0.05% and kept at 30°C for 16 hours with periodical determinations for viable counts, residual H_2O_2, and lactic acid. Under the tested conditions the initial level of contaminated bacteria could be arrested from growing at least for 8, 12, and 16 hours by treating the milk with 0.01, 0.02, and 0.03 per cent of H_2O_2, respectively. Furthermore, when the H_2O_2 concentrations ware limited within the level of 0.03 per cent the added H_2O_2 was completely decomposed within 12 hours without the aid of external catalase and the decomposition time decreased in parallel with the H_2O_2 concentrations. A safer use of H_2O_2 for preserving farm milk temporarily by limiting its concentration has been discussed.

Introduction

The germicidal properties of hydrogen peroxide (H_2O_2) have been tried to be utilized in dairy industry for many years. The early investigations have been reviewed thoroughly by Luck[14] and Roundy [30]. The bactericidal efficiency varies with different organisms, with the bacterial count, the concentration of H_2O_2, the period of time, and the temperature of the treatment. Among the micro-organisms isolated from milk, the coliforms are more susceptible to destruction by H_2O_2 than are spore-forming aerobes and the lactic acid bacteria are intermediate[22,23]. Treatment with H_2O_2 destroys most of the pathogenic organisms but *Mycobacterium tuberculosis* is more resistant than other pathogens. It resisted H_2O_2 concentrations as high as 0.8%[9].

1. This investigation was supported by Korean Traders Scholarship Foundation.

Bovine tubercle bacilli added to milk having 0.08 % of H_2O_2 survived up to 25 hours[27]. Treatment with H_2O_2 to replace pasteurization is therefore recommended only for tubercle-free milk. The works on the possible side effects of this strong oxidizing and bleaching agent on the quality of milk, i.e. flavor, vitamins, milk sugars, proteins, amino acids, casein, enzymes, have also been reviewed by Luck[14]. In general the effects are mild, if pre sent, and no significant damage on milk quality could be expected if the treatment is not unnecessarily strong. Furthermore, there are reports of flavor improvement associated with the H_2O_2 treatment of milk[2,12,19,20,26,34].

Generally, the addition of chemicals to foods has not been accepted. However, H_2O_2 can be destroyed easily, quickly and completery through the addition of catalase, the enzyme which splits H_2O_2 into H_2O and O_2. Preservation of milk with H_2O_2, therefore, been tried in some countries, namely, Italy, France, India, South America and South West Africa. Hydrogen peroxide may be used in the United States also[4] as a desirable bacteriocide[20] in milk used to make certain types of cheese. Recently it has come under consideration by the Food and Agricultural Organization of the United Nations[7], which concluded broadly that the use of any preservative in milk is undesirable and should be adopted only in exceptional circumstances, such as obtain in countries which do not have a highly developed milk handling system. The report further emphasizes that H_2O_2 merely improves the keeping quality of the milk and should not be substituted for pasteurization since, at the recommended concentrations of 0.01 to 0.08% (w/v), certain types of pathogenic organisms are not destroyed. Since pure food grade H_2O_2 is now commercially available and the breakdown products, water and oxygen, have no toxic effect, the only risk should be worried is the incomplete destruction of the added H_2O_2. This may happen through mishandling the treatment by plant workers such as omitting catalase addition. Unpasteurized milk contains variable amounts of catalase, and its content increases parallel with the increase in bacterial count. Hydrogen peroxide is slowly decomposed by the natural catalase of unpasteurized milk[1].

This study was undertaken to find possibility of a safe use of H_2O_2 as preservative of whole milk using a limited amount of H_2O_2 without further addition of catalase. Small amount of H_2O_2 was added to the precontaminated milk and its bactericidal effect and stability were observed.

Materials and Methods

Freshly drawn milk from a local dairy farm was brought to the laboratory and stood both in a refregerator and at the room temperature for various times to obtain different degrees of microbial contamination. 99 *ml* each of the milk samples was then transferred into milk dilution bottles. The original reagent of 30% H_2O_2 (Wato) was pre-diluted with distilled water at various ratios so that an addition of one m*l* of the resulted solutions to the 99 m*l* of milk may give desired concentrations of H_2O_2 in the milk to be treated.

Residual H_2O_2 in the milk after the treatment was analysed by the method of Ferrier et al[8], except that 20% of trichloroacetic acid instead of 1% was used. APHA Standared Methods Agar (Compositions ; tryptone 5 g, yeast extract 2.5 g, glucose 1 g, agar 15 g, distilled water 1000 m*l*) was used for the viable counts.

Results

In the freshly drawn but contaminated farm milk various concentrations of H_2O_2 were added and kept at 30°C for 16 hours with periodical counts for viable cells. As shown in Fig. 1, the initial bacterial contamination around 1×10^5 cells per m*l* was reduced rapidly with the increased level of H_2O_2 added. In the case of 0.01% H_2O_2, however, the initial population could be recovered within eight hours followed by a rapid growth. When the H_2O_2 concentration doubled (0.02%) the milk could be preserved at 30°C at least half a day, whereas higher concentrations were needed to suppress the growth of the survived bacteria for longer than 16 hours.

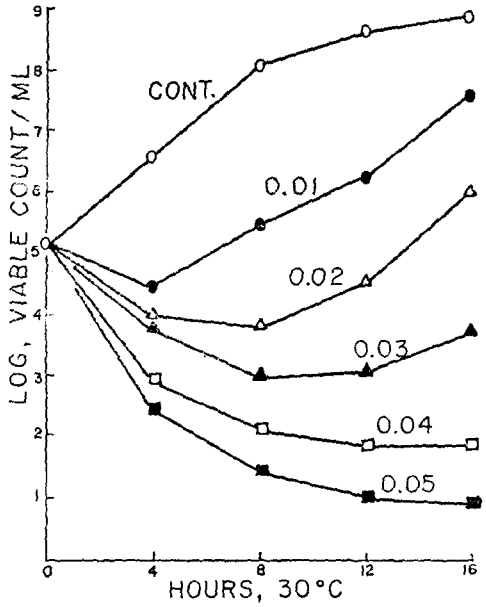

Fig. 1. Effect of Hydrogen Peroxide on the Survival and Growth of Bacteria Contaminated in the Fresh Milk. Hydrogen peroxide was added to the milk to make initial concentrations indicated before incubation.

A complete sterilization could not not be expected even with the 0.05% level of H_2O_2.

The H_2O_2 which had been added in the unpasteurized milk was found to be destroyed rapidly at 30°C (Fig. 2). When the initial concentration of

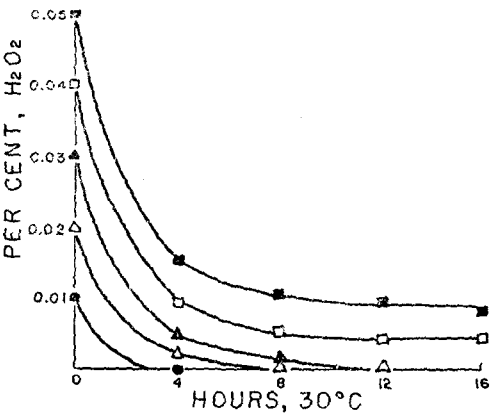

Fig. 2. Stability of H_2O_2 in the Fresh Milk. The same milk as for Fig. 1 was used.
-○- and -△-: Control, -●- and -▲-: with 0.02% H_2O_2.

composition time decreased in parallel with the H_2O_2 concentrations. However, if the concentration exceeded 0.03%, parts of the H_2O_2 undecomposed and remained in the milk for long.

The unstable characteristics of the H_2O_2 in fresh the H_2O_2 was less then 0.03%, a complete decomposition was observed within 12 hours and the de-

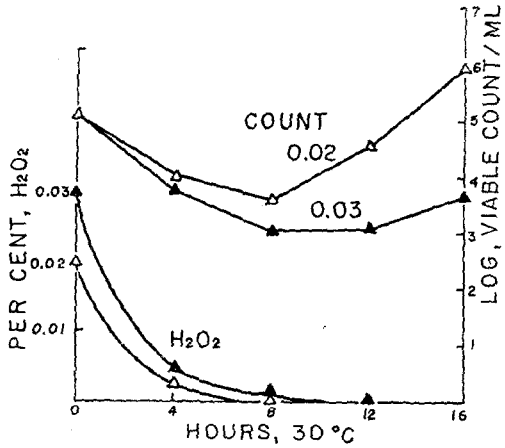

Fig. 3. Relation between Stability and Bactericidal Effect of H_2O_2 in the Fresh Milk. Data adopted from Figs. 1 and 2.

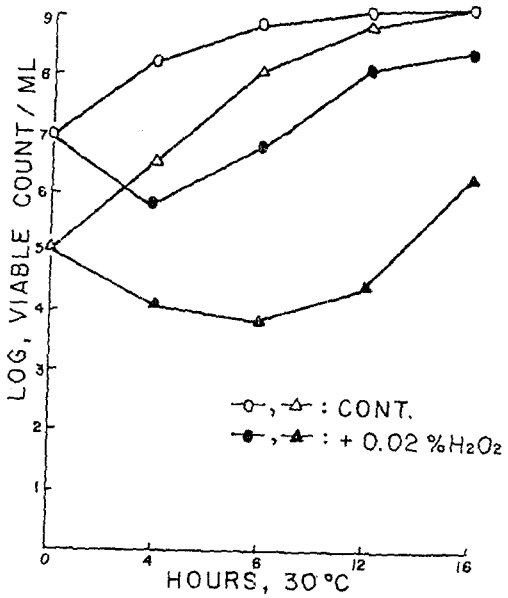

Fig. 4. Effect of Initial Contamination Levels of Milk on Bactericidal Activity of H_2O_2.

milk reflects on the bactericidal effect as shown in Fig. 3. The 0.02% H_2O_2 added to the fresh milk was completely disappeared by eighth hour at 30°C and this was the time from which viable counts started to increase. The same situation was observed in the case of 0.03% H_2O_2 where the shifting occurred after 12 hours of incubation.

The bactericidal effect of H_2O_2 was substantially reduced when the milk was heavily contaminated. As it may be seen in Fig. 4 the growth inhibition by the 0.02% H_2O_2 in the 1×10^7 cells per m*l* contaminated milk (-○-) was released within four hours followed by a rapid growth, while, in the 1×10^5 cells per m*l* milk (-△-), the inhibition lasted at least for eight hours and a slower growth followed after that. Apparently bacterial catalase facilitated decomposition of the added H_2O_2 in the heavily contaminated milk.

The milk having about 2.2×10^5 bacteria per m*l* was treated with 0.02% H_2O_2 and stored at temperatures ranged from 5°C to 30°C (Fig. 5). The results suggest that the level of treatment was enough to preserve the milk for 16 hours at all temperatures tested except 30°C at which bacteria exceeded the initial population during the period.

When the untreated milk was stored at 30°C the initial bacterial population of 1×10^5 cells per m*l* could reach 1×10^8 level in eight hours (Fig. 6). However, the lactic acid production remained unchanged until eighth hour from which a rapid production started. The same delay of acid production behind the population increase was observed in the H_2O_2 treated milk also during the later period of the storage. These findings prove the fact that the early spoilage of contaminated milk is due to the growth of miscellaneous types of bacteria which will gradually be predominated by the lactic acid bacteria.

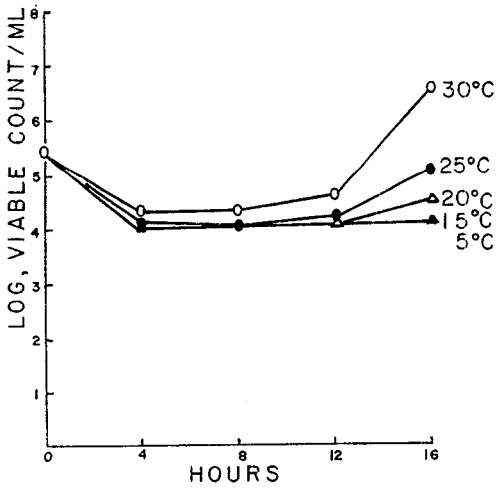

Fig. 5. Effect of Storage Temperatures on Bactericidal Activity of 0.02% H_2O_2 added to the Contaminated Milk.

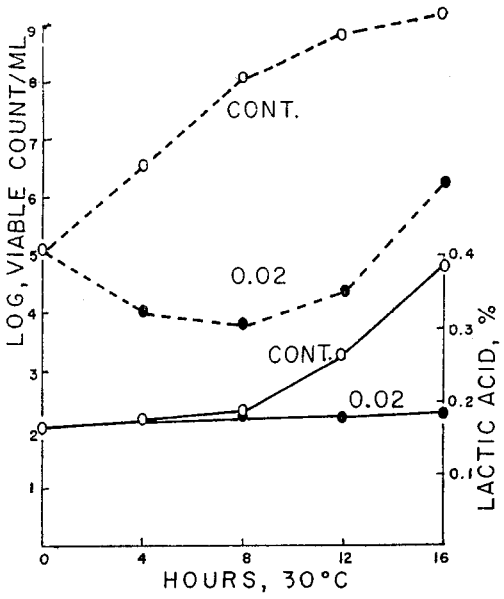

Fig. 6. Relation between Bacterial Growth (broken lines) and Lactic Acid Production (solid lines) in the Milk treated with 0.02% H_2O_2 (-●-) and Untreated (-○-).

Discussion

Satta et al. [31] reported that an addition of 0.05 per cent of 130 vol. H_2O_2 solution (equivalent to 0.02 per cent by weight) arrested the multiplication of bacteria for more than 15 hours at 15°C and 22°C. The rapid growth of bacteria under the same concentration of H_2O_2 in the present case (Fig. 1)

may due to the higher storage temperature of 30° C. We can not expect to remove the contaminated bacteria completely by treating the milk with 0.05 per cent of H_2O_2 (Fig. 1), because even with 0.08 per cent Minaci[17] could reduce only 96.3 per cent of the initial bacterial count at 28°C~30°C.

Catalase has been usually added to the milk after the treatment with H_2O_2 to decompose the residual H_2O_2 into nontoxic compounds of H_2O and O_2 This type of detoxication, however, often accompanies risks of accidental mishandlings by the plant workers. The results appeared in Fig. 2 suggest a safer use of the H_2O_2. When the amount of H_2O_2 added was restricted to the level below 0.03%, the H_2O_2 decomposed completely without the acid of external catalase under the tested conditions. The rapid decomposition of H_2O_2 in the milk may mainly due to the natural catalase liberated from the cells of contaminated bacteria[14]. Luck[14] also pointed heavy metal ions and lactoperoxidase as catalytic agents. In addition, environmental conditions such as temperature and pH effect rate of H_2O_2 decomposition [1,5,6,33]. On the other hand H_2O_2 destroys catalase too[15,35]. According to Molland[16] the decomposition of H_2O_2 by bacteria and the inactivation of the catalase take place simultaneously. The quantity of catalase inactivated is proportional to the quantity of H_2O_2 decomposed. The inactivation of catalase by H_2O_2 is intensified by increasing temperature[18]. The incomplete decomposition of H_2O_2 in the milk when the concentrations exceeded 0.04 % (Fig. 2) may be ascribed to the principles found by these authors. The excess H_2O_2 must have inactivated catalase completely before the H_2O_2 concentrations are further reduced. Therefore, the restriction of the H_2O_2 amount is of paramount importance if the milk is to be preserved by treating with H_2O_2 alone, although other factors such as contamination level, pH, temperature and duration of time are also to be considered carefully.

The long and extensive study has proved H_2O_2 as one of the ideal preservatives. However, the application of H_2O_2 in the dairy industry has not been generally approved. The main reason for this is due to the risk of remaining undecomposed H_2O_2 in the milk. The natural decomposition of added H_2O_2 in the milk may reduce the preservative efficiency of the agent, but may be applied safely for a temporary storage of milk such as the overnight storage of farm milk or during the transportation to the milk processing plants.

요 약

미생물에 의해 오염된 목장우유에 과산화수소를 0.01%에서 0.05% 범위내에서 첨가하고 30°C에서 16시간동안 보관하면서 생균수, 잔여 과산화수소량, 유산의 생성 등을 측정해 보았다. 시험된 조건하에서 0.01, 0.02, 0.03%의 과산화수소 처리는 우유속의 생균수를 각각 8, 12, 16시간동안 시초의 오염정도 이하로 유지시킬 수 있었다. 그뿐 아니라 처리한 과산화수소의 농도를 0.03% 이하로 제한했을 때는 catalase의 첨가없이도 과산화수소가 우유속에서 12시간 이내에 완전히 자연 분해되었으며 그 분해시간은 첨가된 과산화수소의 농도의 감소에 따라 단축되었다. 이런 결과를 토대로 삼아 과산화수소의 첨가량을 줄이므로써 목장우유의 일시적 보관을 위한 보다 안전한 과산화수소의 처리법을 논의하였다.

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