Physical Changes in Buffalo Milk Preserved with Hydrogen peroxide

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Changes in colour, taste, smell and consistency of raw buffalo milk preserved with 0.04, 0.06 and 0.06% hydrogen peroxide kept for 36 hours in glass containers at room temperature were investigated in 10 composite samples obtained from 5—9 she buffaloes of *Nili* and *Ravi* breeds during the summer of 1965 (July-October). Another 5 similar samples treated with 0.06% hydrogen peroxide were kept in aluminium, unglazed earth and glass containers so as to study the effect of the container structure on the above mentioned physical characteristics.

In glass containers 9 of the 10 samples tested showed no change in colour at the 3 levels of the preservative under trial. Coagulation time varied in the 3 concentrations of hydrogen peroxide; out of the 10 samples 9, 4, and 0 coagulating in milk samples preserved with 0.04, 0.06, and 0.08% hydrogen peroxide respectively. Most of the samples whether preserved or not showed no change either in taste or smell until about an hour before curdling. A slight off flavour due to the presence of hydrogen peroxide was, however, noticeable in 0.06 and 0.08% concentrations. Hydrogen peroxide disappeared from all 10, 6 out of 10 and 3 out of 10 samples in 0.04, 0.06, and 0.08% concentrations of the preservative respectively.

In the other containers the position regarding colour, taste and smell was found no different from the one mentioned above. Curdling time ranged between 22—36, 21—35 and 21—34 hours in glass, aluminium and earthen containers respectively, when the unpreserved controls curdled within 13-14 hours of milking. Hydrogen peroxide was not detectable after 12 hours in 4 of the 5 samples kept in aluminium containers and all 5 kept in earthen containers.

INTRODUCTION

West Pakistan annually produces 208.9 million maunds of milk, of which 63.4 per cent comes from she-buffaloes (Haq and Masud, 1966). In spite of this importance, several problems connected with its production, processing and consumption have remained untackled so far. With a view to filling in gaps in the existing knowledge, investigations were organized on the bacterial counts of buffalo milk as affected by different concentrations of hydrogen peroxide used to preserve such milk during the summer months. The results of those investigations have already been reported Aijaz-ul-Haq and Majeed, 1966).

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During the course of investigations referred to above, studies were also made on changes in the physical caracteristics of milk as brought about by hydrogen peroxide. This paper furnishes a resume of those findings. As far as ascertained, the literature does not appear to report such changes in buffalo milk, *vis a vis* their relationship with bacterial counts, under a tropical environment like that of Pakistan (WHO, 1962 ; Karim *et al.*, 1962). It is hoped that the results of this study will help to preserve buffalo milk with hydrogen peroxide effectively and to prevent the loss of a valuable dietary article through spoilage.

REVIEW OF LITERATURE

Depending upon local conditions, different workers have used different concentrations, varying from 0.009 to 0.3 per cent (by weight), of hydrogen peroxide ; but FAO (1957) recommends that the quantity of this preservative should in no circumstance exceed 0.8 gm. per litre of milk (*i.e.*, 0.08 per cent), and it should preferably be 0.1—0.4 gm. per litre (*i.e.*, 0.01—0.04 per cent) of milk intended for fluid consumption. Any quantity in excess of 0.1 per cent influences the constituents of milk unfavourably.

As little information is available on the effects of hydrogen peroxide on the physical characteristics of buffalo milk, the resume furnished here essentially refers to investigations on cow milk. Even in countries like France, Spain, Italy, Nigeria and South Africa, where trial distribution of hydrogen peroxide treated milk has been made, the main product handled was cow milk (WHO, 1962). In Pakistan (Karim, *et al.*, 1962) and India (Iya, 1962), whenever buffalo milk was preserved with hydrogen peroxide, the sole object was the preservation of raw milk in a near normal state, rather than a detailed study of the effects of this preservative on the microbial and physical characteristics of buffalo milk.

Hydrogen peroxide, being colourless and added in small amounts, does not affect the colour of cow milk or its consistency. The taste also remains unaffected provided the preservative has been decomposed completely. As long as it is present, the milk has an objectionable flavour, particularly when its bacterial content is high. Prolonged storage of preserved milk may also show "oxidized" (Nambudripad *et al.*, 1952) and "tallowy" (Krukovsky and Guthrie, 1946) flavours, though subsequent removal of the preservative leaves no detectable flavour. The coagulation time of milk is delayed in accordance with the amount of hydrogen peroxide added. Negretti (1956 as cited by Luck, 1962) added 1 per cent of the preservative and kept milk in good condition upto 39 days at 28°C. Karim *et al.*, (1961, 1962) preserved milk

(from cows and buffaloes) at the rate of 2-3 oz. of 10 per cent hydrogen peroxide per 100 lb. of milk at 86-120°F and prevented coagulation for 12-36 hours.

Excepting ascorbic acid (which is affected seriously with hydrogen peroxide ; but milk is not an important source of this vitamin), all other vitamins remain intact. Gregory *et al.*, (1961) treated milk with 0.05 per cent hydrogen peroxide for 8 hours at 24°C. and found that carotene, vitamin A, xanthophyll, tocopherol, thiamine, riboflavin, nicotinic acid, pyridoxine, calcium pantothenate and biotin remained unaffected. As regards casein in higher concentrations of hydrogen peroxide oxidize proteins and aldehydes, but dilute solutions do not show this effect (Luck, 1962). The same holds true of changes in the *B*—lactoglobulin of milk. Among amino acids, some are very susceptible to hydrogen peroxide, particularly cysteine, cystine and methionine ; while others like tyrosine and trytophane are also oxidized.

Giolitti (1949 as cited by Luck, 1962) has reported no changes in lactose, fat, total nitrogen and pH upto a concentration of 0.04 per cent of this preservative. Enzymes of milk are affected to a certain extent. Cimino (1945 as cited by Luck, 1962) has reported that concentrations of 0.08 to 0.12 per cent at freezing temperatures, as well as at 20 to 30°C., did not affect amylase, lipase, tryptase and phosphatase, but nearly destroyed peroxidase, catalase, and reductase.

The Expert Group of FAO (1957) has stated that, of the various preservatives available for milk, the only one permissible is pure grade hydrogen peroxide. Among other things, they have recommended that investigations should be carried out to evaluate more precisely the changes in the quality of milk in relation to human health and nutrition. Kon (1964) has recently stated that, while striving for perfection, people should accept treatment with hydrogen peroxide, intelligently applied when need demands, as a timely and useful measure.

MATERIAL AND METHODS

In all, 15 composite samples (comprising 60 samples) of raw milk from she-buffaloes of *Nili* and *Ravi* breeds were collected for investigations spreading over four summer months (July-October, 1965). Every composite sample contained milk from 5 to 9 she-buffaloes and was taken within 15-20 minutes of milking from the pail used for routine bulking Aijaz-ul-Haq and Majeed, 1966). Of these, 10 composite samples were used to investigate the effects of different concentrations of hydrogen peroxide on the physical characteristics of milk ; while the other 5 composite samples were preserved with hydrogen peroxide to investigate the changes in physical characteristics as affected by

the material of the containers, *viz.*, glass, aluminium and ungiazed earth. These containers were employed because they are in vogue all over the country and because aluminium is highly compatible with hydrogen peroxide. The glass containers were sterilized, but those of aluminium and earth were not sterilized purposely : they were used after cleaning with soap and hot water, as is the practice in an average home.

All composite samples were processed in the laboratory within 20-35 minutes of milking. Every composite sample was divided into four samples, each measuring 100 ml. One sample was kept aside to serve as control, while the remaining three samples were treated with hydrogen peroxide (30 per cent by weight), to yield final concentrations of 0.04, 0.06 and 0.08 per cent of the preservative. To study physical changes in milk after 12, 24, and 36 hours, milk was kept in 12 separate flasks (3 for control and 9 for hydrogen peroxide treated samples), so as to minimize contamination from the handling of the same flask every twelve hours. The standard plate counts were determined on Milk Protein Hydrolysate Medium, after incubation at 32°C. for 48 ± 3 hours, as described by Aijaz-ul-Haq and Majeed (1966). In the case of the glass, aluminium, and earthen containers, the concentration of hydrogen peroxide was kept constant at 0.06 per cent.

The colour, taste, smell, and coagulation time of untreated and treated milk were evaluated with the help of sensory tests, while the presence of hydrogen peroxide was determined with the help of vanadium pentoxide (WHO, 1962).

RESULTS AND DISCUSSION

(1) Physical Changes in Milk Preserved in Glass Containers

The physical characteristics of raw buffalo milk preserved with hydrogen peroxide were studied. The changes which took place in milk characteristics on storage upto 36 hours are given individually below.

(*a*) *Colour.*—The colour of buffalo milk remained unchanged in the control as well as hydrogen peroxide treated samples stored upto 36 hours. In one composite sample, however, the upper surface of the scum of coagulated milk in the control sample (which coagulated 11 hours after milking) and the sample preserved with 0.04 per cent hydrogen (which coagulated after 34 hours) showed a light yellow colour. Other samples of this composite sample, which had been preserved with 0.06 and 0.08 per cent hydrogen peroxide, did not coagulate upto 36 hours, nor showed yellowish coloration. The light yellow colour most probably resulted from the contamination of milk with an organism like *Pseudomonas synxantha*, which was killed or inactivated by 0.06 and 0.08 per cent hydrogen peroxide.

(*b*) *Taste and smell.*—Most samples, whether untreated or treated with hydrogen peroxide, showed no change in taste or smell until the time of their coagulation. In 20 per cent cases, however, milk turned sour a few hours before curdling. Among such samples, three turned sour but did not coagulate upto 36 hours. As soon as a sample showed coagulation, the taste of milk turned sour and so did its smell. As regards the effect of hydrogen peroxide on the taste and smell of milk, all samples remained nearly normal upto the time of curdling. A slight off-flavour developing from the addition of the preservative was almost unnoticeable with 0.04 per cent, noticeable with 0.06 per cent and readily noticeable with 0.08 per cent concentration.

(*c*) *Consistency and coagulation time.*—The consistency of milk in all samples started increasing about an hour and a half before complete coagulation. The time of coagulation in the control samples varied from 11 to 14 hours after milking. In the samples treated with 0.04 per cent hydrogen peroxide, one sample did not coagulate upto 36 hours, while the coagulation time in the other nine samples varied from 22 to 34 hours. In the samples treated with 0.06 per cent hydrogen peroxide, only four out of the ten samples coagulated within 36 hours, the time of their coagulation varying from 22 to 36 hours. In the samples treated with 0.08 per cent hydrogen peroxide, no sample showed coagulation upto 36 hours.

The SPCs of milk within 3 hours of its coagulation were arranged into three interval groups in which coagulation occurred most frequently, *viz.*, 11 to 14 hours, 21 to 23 hours and 33 to 36 hours (Aijaz-ul-Haq and Majeed, 1966). It was found that buffalo milk curdling at the end of about half a day, one day and one and a half day had SPCs of 82, 50 and 37 million per ml. respectively. There was a wide variation in counts when the samples showed complete coagulation, *i.e.*, from 10 to 262 million per ml., but the usual range was 30-100 million. The results show that although the curdling and souring of milk depend on its microbial contamination, species and strains of microbes, container material and additives also play an important role. The maximum amount of acid developed in milk varies with different species, and even with different strains of the same species. Likewise, preservatives and flavouring agents can induce chemical changes and cause souring and coagulation. A similar process is seen if milk is stored in rusty metallic vessels or those of unglazed earth of uncertain sanitation. The SPCs of curdled cow milk have been shown to vary within wide limits, though the usual range for souring with *Streptococcus lactis* has been given at 30-90 million per ml. (Foster *et al.*, 1957). When raw milk develops acidity and starts curdling, lactose ($C_{12}H_{22}O_{11}$) hydrolyses into lactic acid ($C_2H_2O_2$).

(d) Disappearance of hydrogen peroxide.—With a view of finding out the time of disappearance of hydrogen peroxide from the samples of milk preserved with different concentrations of preservative, all samples were tested with the help of vandium pentoxide regularly after 12, 24, and 36 hours of milking. The summary of the results is furnished in Table 1.

TABLE 1. *Disappearance of Hydrogen peroxide from preserved milk.**

Concentration of Hydrogen peroxide	Number of samples of milk preserved with hydrogen peroxide giving				
	Negative results after			Positive results after 12-36 hrs.	Total
	12 hrs.	24 hrs.	36 hrs.		
0.04	7	2	1	0	10
0.06	3	2	1	4	10
0.08	0	2	1	7	10

*Hydrogen peroxide was added 25 minutes after milking.

Table 1 shows that hydrogen peroxide used to preserve buffalo milk in a concentration of 0.04 per cent disappeared in all samples within 36 hours. This concentration inactivated the smallest number of microbes and allowed milk to remain uncurdled between 22 and 34 hours in 10 per cent samples. In the case of milk preserved with 0.06 per cent concentration, hydrogen peroxide persisted in four and disappeared in six samples. This concentration inactivated a good number of microbes and allowed milk to remain uncurdled between 22 and 35 hours in 40 per cent samples. In the case of milk preserved with 0.08 per cent concentration, hydrogen peroxide persisted in seven and disappeared in three samples. This concentration inactivated the highest number of microbes and did not allow milk to coagulate upto 36 hours.

The vanadium pentoxide test for the presence of hydrogen peroxide being very sensitive (detects less than 0.008 per cent of the preservative), it gives a good indication of the disappearance of hydrogen peroxide from milk. The earlier the hydrogen peroxide disappears, the lesser is the inactivation of the microbes in milk and the earlier is its spoilage through coagulation. The average figures of SPCs of 40.2, 20.0, and 13.4 million per ml. after 36 hours' storage in the respective concentrations of 0.04, 0.06 and 0.08 per cent of hydrogen peroxide bear out this view.

(2) Physical Changes in Milk Preserved in Glass, Aluminium and Earthen Containers

The SPCs, as well as the physical characteristics of milk preserved with hydrogen peroxide in the three different kinds of containers were studied. The changes which took place in milk characteristics on storage upto 36 hours are dealt with individually below :

(*a*) *Colour.*—The colour of buffalo milk remained unchanged upto 36 hours in the control as well as 0.06 per cent hydrogen peroxide treated samples stored in the glass, aluminium, and earthen containers. These results indicated that 0.06 per cent hydrogen peroxide concentration was good enough for preserving buffalo milk even in the unsterilized aluminium and unglazed earthen containers in so far as the colour of buffalo milk was concerned.

(*b*) *Taste and smell.*—In all samples kept as control, taste and smell became sour within 12 hours and milk curdled completely at 13 to 14 hours after milking. In the samples preserved with 0.06 per cent hydrogen peroxide in sterilized glass flasks, the taste and smell remained nearly normal upto 12 hours in two samples and upto 24 hours in three samples. In the case of similarly preserved milk in the aluminium containers, the taste and smell turned sour within 12 hours in one sample, between 12 and 24 hours in three samples, and within 24 to 36 hours in one sample. In the case of milk preserved in earthen containers, the taste and smell position was the same as in the case of the aluminium containers. Under the conditions of this experiment, taste and smell changed appreciably when the SPCs were in the vicinity of 27 million per ml. in the case of control samples and in the vicinity of 35 to 52 million per ml. in the case of preserved samples, the two extremes being 13 million and 68 million per ml. Excepting a slight off-flavour developing as a result of the addition of hydrogen peroxide, no other deterioration was noticed.

(*c*) *Consistency and coagulation.*—All samples of milk thickened in consistency about an hour and a half before complete curdling. Whereas unpreserved milk in sterilized glass containers curdled at 13 to 14 hours after milking, that preserved with 0.06 per cent hydrogen peroxide in the sterilized glass flasks and unsterilized aluminium and earthen containers curdled after 22 to 36 hours, 21 to 35 hours and 21 to 34 hours respectively. This time of coagulation was longer by a couple of hours than that in the first set of experiments as a result of comparatively low atmospheric temperature. Though the time of coagulation in the case of the aluminium and earthen containers was about the same, the final bacterial content after 12, 24 or 36 hours was lower in the aluminium than in the earthen containers, showing that there are more chances of early curdling of milk kept in the earthern than in the aluminium containers.

(*d*) *Disappearance of hydrogen peroxide.*—In the samples preserved in the sterilized glass flasks, hydrogen peroxide disappeared within 12 hours in four samples, and between 12 and 24 hours in one sample. In the case of samples stored in the unsterilized aluminium containers, the disappearance was similar to that observed for milk in the glass flasks. In the case of the earthen containers, hydrogen peroxide disappeared within 12 hours in all samples. Thus the unglazed earthen containers are not as suitable for preserving buffalo milk with hydrogen peroxide as those made of glass and aluminium.

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