PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 2 (1): 36-42, 2003

Study on the Preservation of Raw Milk with Hydrogen Peroxide (H_2O_2) for Rural Dairy Farmers

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Abstract: The experiment was conducted to judge the feasibility of hydrogen peroxide as milk preservation. The experiment milk sample collected from Bangladesh Agricultural University Dairy Farm and were divided into seven portions. Six portions were preserved with 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2. The reaming portion was preserved without H_2O_2 and considered as control sample. Some physical and chemical parameters were measured from all samples just before preservation and then regularly after certain time interval up to spoilage. From the results of physical and chemical tests, it was observed that keeping quality of milk samples with H_2O_2 increased significantly when compared with untreated milk samples. It was concluded that 0.04 to 0.05% H_2O_2 is enough to preserve milk sample up to 24 hours.

Key words: Raw milk, H_2O_2, treated milk, milk preservation, preservation period

Introduction

Milk is the most important food item for human beings. Both children and adults require it for maintaining their normal body. Milk contains on average 87.25% water 3.80% fat, 3.50% protein, 4.80% of lactose and 0.65% minerals according to (Eckles *et al*., 1951). Besides milk contains considerable amount of fat-soluble vitamins (Vit A, D, E, K) and water-soluble vitamin (Vit B complex and vit C).

Milk is very nutritious, but it is also a good media for growth of various microbes. The presence of undesirable bacteria in milk may causes deterioration of flavor or physical appearance and also may be the causes of producing disease in human beings. Souring of milk, discoloration, gassiness and many other defects are caused by the presence of different types of microorganisms. In our country, majority of the dairy farmers have no ability to install cold room or to buy refrigerator. Similarly heated milk is not generally accepted by the public in the market. Another alternative way is to preserve milk with chemical preservatives.

Recently scientists are using various milk preservatives (H_2O_2, $NaHCO_3$, ethanol, boric acid) to overcome this problem (Al-Dabbagh *et al*., 1984; Dirar, 1975; Hossain, 1989). So, it is very important to carry out research work for developing a suitable method of milk preservation for our rural farmers. So, the present study was under taken to preserve milk samples with hydrogen peroxide.

Materials and Methods

The experiment was conducted at Dairy Technology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University, Mymensingh, Bangladesh for the period of February to March 1999.

Source of Milk: Whole milk was collected from Bangladesh Agricultural University Dairy Farm. Suggestions were given to milkers before milking the cows for maintaining all hygienic measure.

Experimental procedures: The collected milk samples, after thorough mixing was divided into seven equal parts. Out of the seven parts, one part was kept as untreated milk (fresh) and six portions were treated with 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% of H_2O_2 (30 W/v). Plastic containers were used to milk preservation. The parameters used to monitor the physical and chemical quality of milk were determined initially just before adding hydrogen peroxide and then after adding the preservatives until the milk samples were spoiled.

Physical parameters (Organoleptic test): Organoleptic test was performed nasally, visually to observe flavour , colour and texture, specific gravity test was performed by using Quevennae lactometer according to the method described by Aggorwala and Sharma, 1961.

Chemical Parameters: Fat test was done according to Babcock Fat test method as described by Aggarwala and Sharma, 1961. Protein test was done by formal titration method (Bennenberg *et al*., 1949). Acidity test was done as per method described by A. O. A. C, 1971. Methylene blue reduction test was performed according to American Public Health Association (A. P. H. A), 1967.

Statistical analysis: Data were analyzed using completely Randomized Design as per (Steel and Torrie, 1980). Analysis of variance test was done to find the statistical difference (Significant or not) between the different treatments and to make a comparison between different treatment means LSD value was calculated.

Results and Discussion

Physical parameters:

Flavour : Flavour of hydrogen peroxide treated and untreated milk samples are presented in Table 1. On

Table 1: Flavour quality of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments						
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%
0-8	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
12	Slight Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
14	Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
16	Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
17	Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
18	Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
19	Bitter	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
20	Bitter	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
21	Bitter	Slight Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
22	Bitter	Slight Sour	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
23	Bitter	Slight Sour	Slight Sour	Slight Sour	Pleasing	Pleasing	Pleasing
24	Bitter	Sour	Slight Sour	Slight Sour	Pleasing	Pleasing	Pleasing
25	Bitter	Sour	Slight Sour	Slight Sour	Pleasing	Pleasing	Pleasing
26	off flavor	Bitter	Slight Sour	Slight Sour	Slight Sour	Pleasing	Pleasing
27	off flavor	Bitter	Sour	Sour	Slight Sour	Slight Sour	Pleasing
28	off flavor	Bitter	Sour	Sour	Sour	Slight Sour	Slight Sour
29	off flavor	Bitter	Bitter	Bitter	Sour	Sour	Slight Sour
30	off flavor	Bitter	Bitter	Bitter	Bitter	Sour	Slight Sour
31	off flavor	Bitter	Bitter	Bitter	Bitter	Bitter	Sour
32	off flavor	Bitter	Bitter	Bitter	Bitter	Bitter	Sour

Table 2: Flavour score of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments							LSD	Level of Significance
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%		
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
4	97.77	100.00	100.00	100.00	100.00	100.00	100.00		
8	91.11	98.88	98.88	100.00	100.00	100.00	100.00		
12	72.22	92.22	97.77	98.88	100.00	100.00	100.00		
16	41.11	82.22	96.66	98.88	98.88	98.88	100.00		
20	32.22	82.22	86.66	86.66	87.77	87.77	98.88		NS
24	26.66	66.66	74.44	74.44	78.88	83.33	93.33		
28	22.22	34.44	41.11	45.55	52.22	54.44	65.55		
32	16.66	25.55	33.33	41.11	41.11	42.22	51.11		
Mean ± SD	55.55± 34.48	75.79± 28.22	80.98± 26.23	82.83± 24.01	84.31± 22.73	85.18± 21.98	89.87± 18.37		

* NS = Non Significant

Table 3: Colour quality of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments						
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%
0-8	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
12	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
14	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
16	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
17	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
18	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
19	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
20	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
21	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W	Y.W
22	Bleached	Bleached	Y.W	Y.W	Y.W	Y.W	Y.W
23	Bleached	Bleached	Bleached	Bleached	Y.W	Y.W	Y.W
24	Bleached	Bleached	Bleached	Bleached	Y.W	Y.W	Y.W
25	Bleached	Bleached	Bleached	Bleached	Bleached	Y.W	Y.W
26	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Y.W
27	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached
28	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached
29	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached
30	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached
31	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached
32	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached	Bleached

Table 4: Colour quality of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments							LSD	Level of Significance
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%		
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
4	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
8	96.66	96.66	97.77	100.00	100.00	100.00	100.00		
12	67.77	88.88	96.66	97.77	98.88	100.00	100.00		
16	58.88	83.33	93.33	93.33	94.44	94.44	94.44		
20	53.33	75.55	88.88	91.11	91.11	94.44	94.44		NS
24	48.88	60.00	67.77	70.00	78.88	88.88	91.11		
28	42.22	52.22	60.00	63.33	66.66	66.66	70.00		
32	33.33	40.00	52.22	55.55	58.88	62.22	62.22		
Mean±SD	66.78± 25.94	77.40± 22.07	84.07± 18.78	85.67± 17.68	87.65± 15.75	89.62± 14.81	90.24±14.19		

* NS = Non significant

the other hand changes in flavour score during study period in different types of milk samples are shown in Table 2. It is evident that flavour of fresh milk (Without H_2O_2), 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples were acceptable up to 8, 20, 22, 25, 26 and 27 hours respectively. After that time flavour was becoming unacceptable. This result showed that hydrogen peroxide is effective for controlling the flavour of milk. The total flavour score of thirty two hours study for fresh, 0.01, 0.02,0.03, 0.04, 0.05 and 0.06% hydrogen peroxide preserved milk samples were 55.55 ± 34.48, 75.79 ± 28.22, 80.98 ± 26.23, 82.83 ± 24.01, 84.31 ± 22.73, 85.18 ± 21.98 and 89.87 ± 18.37 respectively. From the Table 2 it is evident that flavour score of untreated milk (fresh) samples decreased very rapidly, but the decrease of flavour score was slow in case of H_2O_2 treated milk samples. For untreated milk samples flavour score started to decrease from four hours of preservation. But for 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 preserved milk samples, the score started to decrease from 8, 8, 12, 16, 16 and 20 hours of preservation samples for long time when compared with untreated milk samples at ordinary room temperature 23-25 ^{O}C). The results of present experiment are in agreement with the findings of several researchers (Hami, 1973; Dirar, 1975; Ambadkar and Lembhe, 1994). Whom reported that H_2O_2 is an effective chemical for preserving milk samples under ordinary room temperature.

Colour: The colour of untreated and H_2O_2 treated milk samples are shown in Table 3. The colour of all samples was yellowish white during starting the experiment. From the table it is evident that for untreated, 0.01,0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples, colour was normal up to 8, 21, 22, 22, 24, 25 and 26 hours respectively and after which colour become bleached. Colour deterioration was very rapid in untreated milk, followed by 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% hydrogen peroxide treated milk samples. Score card for colour are shown in Table 3 and 4 showed that total colour score during the whole experimental period was 66.78 ± 25.94, 77.40 ± 22.07, 84.07 ± 18.78, 85.67 ± 17.68, 87.65 ± 15.75, 89.62 ± 14.81 and 90.24 ± 14.19 respectively. Colour score deteriorated with increasing storage time of milk sampled. Deterioration was rapid in increased milk samples followed by 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples. The result of this experiment agrees with the findings of Kang *et al.*, 1983. According to them H_2O_2 is an effective chemical for milk preservation and addition of 0.02% H_2O_2 with milk protects from spoilage up to 12 hours at 20-30 ^{O}C at room temperature.

Texture: The texture of all milk samples are shown in Table 5. The normal texture of milk is stated as "free flowing liquid". From this Table it is evident that texture of untreated, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples were normal up to 8, 21, 25, 27, 28, 29 and 31 hours respectively. There after samples become clotted. Curd was seen in untreated milk samples and in cases of H_2O_2 treated milk samples some proteolysis was noticed. Similar types of results were obtained by Dirar, 1975 who observed that spoilage of raw milk was due to normal souring but H_2O_2 treated milks were spoiled by proteolytic and sweet curdling changes. The author also stated that H_2O_2 was most effective against souring bacteria. The score card of texture of untreated and H_2O_2 treated milk samples are presented in Table 6. Average texture score was 60.11 ±32.34, 78.39 ± 26.19, 83.94 ± 21.99, 85.67 ± 21.73, 88.63 ± 14.10, 90.36 ± 13.79 and 93.20 ± 10.57% respectively for untreated, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples respectively. Significant difference was observed within texture score of different types of milk sample. Texture deterioration was rapid in untreated milk samples followed by 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples. The result of deterioration agrees with the findings of Hossain, 1989; Gupta, 1986. They found that lactic acid production was rapid in untreated milk samples. This might have a cause of rapid changes of texture in untreated milk.

Chemical Parameters:

Acidity Test: The acidity percent of untreated and H_2O_2 treated milk samples are shown in (Table 7). The average acidity of untreated, 0.01, 0.02, 0.03, 0.04, 0.05, and 0.06% H_2O_2 treated milk samples were 0.404 ±0.18, 0.233 ±0.08, 0.211 ±0.06, 0.198 ± 0.04, 0.192 ± 0.04, 0.188 ±0.04 and 0.177 ± 0.03% respectively. Significant difference was found with in the acidity of different types of milk samples. The result of acidity test are in agreement with several research workers. Siegenhalar, 1976 reported that, in tropical condition it is possible to preserve milk for at least 24 hours with addition of 0.06 to 0.08% of H_2O_2 in raw milk. From another experiment, Park and Pack, 1977 found that growth of contaminating bacteria in raw milk could be checked for at least 8, 12 or 16 hours by treatment with 0.01, 0.02 or 0.03% H_2O_2 respectively. Similar types of results were also observed by (Hossain, 1989; Kang *et al.*, 1983; Gupta *et al.*, 1986; Ambadkar *et al.*, 1991; Abd-El-Hday *et al.*, 1995). It is well known that the acidity in milk is developed due to the break down of milk sugar lactose into lactic acid by the fermentative effect of acid producing bacteria. H_2O_2 prevents bacterial fermentation by inhibiting the growth of acid producing bacteria in milk. This might be the cause of lower acidity in H_2O_2 treated milk samples.

Table 5: Texture quality of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments						
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%
0-8	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
12	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
14	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
16	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
17	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
18	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
19	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
20	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
21	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
22	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
23	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
24	Curd	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
25	Curd	Clotted	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
26	Curd	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing
27	Curd	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing
28	Curd	Proteolysis	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing
29	Curd	Proteolysis	Clotted	Slight clotted	Slight clotted	Free flowing	Free flowing
30	Curd	Proteolysis	Proteolysis	Clotted	Slight clotted	Free flowing	Free flowing
31	Curd	Proteolysis	Proteolysis	Clotted	Clotted	Slight clotted	Free flowing
32	Curd	Proteolysis	Proteolysis	Proteolysis	Clotted	Clotted	Slight clotted

Table 6: Texture score of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments							LSD	Level of Significance
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%		
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
4	100.00	100.00	100.00	100.00	100.00	100.00	100.00		
8	96.66	96.66	98.88	98.88	98.88	100.00	100.00		
12	73.33	96.66	96.66	98.88	98.88	98.88	98.88		
16	46.66	90.00	93.33	96.66	95.55	96.66	97.77		
20	38.88	83.33	86.66	86.66	86.66	93.33	93.33	6.59	**
24	33.33	66.66	80.00	83.33	84.66	90.00	93.33		
28	28.33	38.88	66.66	73.33	73.33	75.55	90.00		
32	23.33	33.33	33.33	33.33	60.00	60.00	66.66		
Mean± SD	60.11± 32.34	78.39± 26.19	83.94± 21.99	85.67± 21.73	88.63± 14.20	90.36± 13.79	93.20± 10.57		

** P< (0.01)

Table 7: Average acidity of control and different proportions of hydrogen peroxide treated milk samples during preservation period

Hour	Treatments							LSD	Level of significance
	Control	0.01%	0.02%	0.03%	0.04%	0.05%	0.06%		
0	0.14	0.14	0.14	0.14	0.14	0.14	0.14		
4	0.151	0.143	0.141	0.141	0.14	0.14	0.14		
8	0.171	0.151	0.15	0.146	0.141	0.14	0.14		
12	0.24	0.161	0.158	0.151	0.145	0.143	0.14		
14	0.256	0.166	0.163	0.153	0.148	0.148	0.143		
16	0.271	0.171	0.168	0.158	0.155	0.155	0.148		
17	0.283	0.171	0.168	0.161	0.155	0.155	0.151		
18	0.295	0.175	0.171	0.166	0.16	0.158	0.153		
19	0.305	0.176	0.173	0.171	0.165	0.161	0.156		
20	0.333	0.18	0.176	0.176	0.17	0.166	0.158		
21	0.351	0.191	0.185	0.181	0.175	0.171	0.163		
22	0.381	0.208	0.195	0.191	0.185	0.176	0.166	0.012	**
23	0.41	0.226	0.21	0.203	0.195	0.186	0.17		
24	0.44	0.243	0.22	0.208	0.198	0.196	0.18		
25	0.473	0.253	0.233	0.223	0.21	0.206	0.19		
26	0.503	0.265	0.243	0.231	0.221	0.218	0.20		
27	0.538	0.283	0.253	0.236	0.233	0.23	0.211		
28	0.581	0.298	0.261	0.243	0.241	0.236	0.211		
29	0.621	0.326	0.278	0.251	0.246	0.245	0.228		
30	0.670	0.358	0.296	0.265	0.256	0.25	0.235		
31	0.713	0.401	0.323	0.28	0.266	0.261	0.237		
32	0.773	0.44	0.353	0.298	0.283	0.276	0.242		
Mean ± SD	0.404±0.18	0.233± 0.08	0.211± 0.06	0.198± 0.04	0.192± 0.04	0.188±0.04	0.177±0.03		

** P < (0.01)

Table 8: Observation of average positive methylene blue reduction time under various treatments

Nature of Treatments	Observation time														
	8.30am	9.30am	10.30am	11.30am	12.30pm	1.30pm	2.30pm	3.30pm	4.30pm	5.30pm	6.30pm	7.30pm	8.30pm	9.30pm	10.30pm
Control	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
.01%	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
.02%	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
.03%	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
.04%	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
.05%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
.06%	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Methylene Blue Reduction Test: Methylene blue reduction test was conducted to get an idea about the bacterial population in untreated and hydrogen peroxide treated milk samples. The results methylene blue reduction test are shown in Table 8. From the Table it is evident that colour reduction time of methylene blue test was 8, 10, 11, 12,12, 14 and 14 hours for untreated, 0.01, 0.02, 0.03, 0.04, 0.05 and 0.06% H_2O_2 treated milk samples respectively. Colour reduction time of methylene blue depends on the amount of micro-organisms present in milk. Higher the number of micro-organisms the lower will be the reduction time and vice versa. Colour reduction time of untreated milk of samples was lower than H_2O_2 treated samples. Bacterial population in raw milk multiplies rapidly and hence the colour reduction time was less in untreated milk samples. On the other hand hydrogen peroxide kills or inhibits the growth of bacterial population and for this reason colour reduction time was less in H_2O_2 treated milk samples. It is also evident that reduction time depends on the amount of H_2O_2 used for milk preservation. The result of this experiment agrees with the findings of Hossain, 1989 who found that colour reduction time for H_2O_2 treated milk samples were less than untreated milk samples. The result of methylene blue test also indicates that H_2O_2 is an effective chemical for milk preservation. The result also confirms the work of several researchers whom reported that H_2O_2 is an economic and effective means of short term alternative milk preservation system for developing countries.

From the results of the above mentioned parameters it is now clear that H_2O_2 is effective to kill or inhibit the growth of bacteria in raw milk and can be used as a preservative of milk under rural condition of Bangladesh. Addition of 0.03 to 0.04% H_2O_2 with raw milk is enough to preserve milk for up to 22 to 24 hours.

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