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Article

Effect of hydrogen peroxide (H_2O_2) on shelf life and bacterial population of raw milk

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Abstract: The present study was conducted to assess the feasibility of hydrogen peroxide (H_2O_2) for milk preservation and also to investigate the effects of H_2O_2 on growth of bacteria population in milk. Milk samples were collected from Bangladesh Agricultural University dairy farm and preserved with 0.12% H_2O_2 of 10% concentration on the basis of volume of milk and milk without H_2O_2 addition treated as control sample. Parameters used to monitor the shelf life of milk were organoleptic and chemical tests. All milk samples were kept in glass container at room temperature (28-31°C) in the laboratory. Organoleptic parameters (color, flavor, texture, taste) and chemical tests (acidity %, COB test and pH) were done every one hour interval until spoilage. Standard Plate Count (SPC) of the samples was done two times, initially and after spoilage of milk. Acidity% increased gradually in all sample but this increase was rapid in control sample than H_2O_2 treated sample. From COB test it was found that raw milk sample gave positive result at 10th hour but 0.12% H_2O_2 treated sample (after 20 min of milking) and 0.12% H_2O_2 treated sample (after 3 hrs. of milking) gave positive result 14 and 15th hour respectively. From the result it was observed that shelf life of milk with H_2O_2 increased. From the SPC result it was found that the bacterial growth rate is very high in control milk sample compare to H_2O_2 treated milk sample. Significant difference ($p<0.01$ was found in case of bacterial growth rate of the milk samples. The results of this experiment indicated that H_2O_2 be used as an effective preservative for prolonging milk preservation. So H_2O_2 can be used as an effective milk preservative for prolonging milk storage in environmental condition.

Keywords: milk; H_2O_2; milk preservation; SPC; bacterial growth

1. Introduction

Milk is an important source of nutrients to human and animals. It is meant to be the first and the only food for the offspring of mammals as is almost complete food. Almost 87% of milk is composed of water and the remaining part comprises total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body (Hossain *et al.,* 2017). Other milk ingredients include immunoglobulins which protect the newly born against a number of diseases (Pandey and Voskuil, 2011). Livestock and its products provide direct cash income, since they are living for rural farmer and are critical to agricultural intensification where it provides power and manure as fertilizer and fuel. In Bangladesh milk and milk products are not sufficient for human consumption. Maximum amount of available milk is used in liquid form. According to Department of Livestock Services (DLS, 2020) the 106.80 Lakh metric ton milk is produced in 2019-2020. In our country maximum milk is produced in rural areas. Most of the cows in our country are indigenous type (Rahman *et al.,* 2016; Islam *et al.,* 2016). Although milk is very

nutritious food but it is very perishable product and its quality can be deteriorated very quickly if it does not keep properly (Akter *et al.,* 2020). The quality deterioration of milk not only affects the flavor and nutrition of milk, but also affects the keeping quality. Milk is also an excellent medium for the growth of a large variety of microorganisms (Hassan *et al.,* 2018). There are so many sources for microbial contamination of milk viz. cow's udder, body of the cows, bedding materials, foods, floor flies, insects, rodents, water supply, milk utensils, air, manure, transportation, atmosphere etc. So care must be taken from initial stage to produce quality milk and milk products.

As a result, it may cause deterioration of flavor of physical appearance and also may be the cause of producing disease in human being. The keeping quality of milk is important for the dairy industry both in the developed and underdeveloped countries. It is important that milk, after having been drawn is cooled rapidly. Subsequent transport at temperatures of not more than 4ºC is necessary to avoid the increased growth rate of psychotropic bacteria. After delivery at the dairy, the milk should be cooled again to 4ºC (Stadhouder, 1982). Milk is an ideal habitat for the growth and multiplication of microorganisms due to its nutritional constitution. Milk is the nutrient-rich liquid contains protein, carbohydrate, unsaturated fat, mineral and vitamins. All these components support the growth of many forms of bacteria (Sarker *et al.,* 2020). Raw milk aseptically drawn from a healthy animal usually contains a few bacteria (Omer *et al.,* 2008; Sarker *et al.,* 2018). Since raw milk quality is very important for the quality milk and milk product made of it therefore, quality of raw milk should be under control. So, safety of dairy products with respect to food-borne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Asaminew and Eyassu, 2011).

The use some local techniques to preserve cow milk in raw condition during transportation by putting water hyacinth leaves or date leaves etc. in milk. But this technique does not give satisfactory results and also makes the quality of milk in an unhygienic condition. Sometimes this technique brings changes in the quality of milk in raw condition which makes it unsuitable for consumption.

The preservation of milk and milk products should be secured through improved production and processing methods rather than through the addition of preservatives; secondly, milk and dairy products are basic foodstuffs, and as such should be free. Various milk preservatives, such as H_2O_2, $NaHCO_3$, ethanol, boric acid etc. are used to retards the growth of microorganisms. Out of the various chemical substances, H_2O_2 is recognized as a good preservative in many countries of the world. FAO/WHO strongly discourages the preservation of milk by chemical means, except the application of H_2O_2 at moderate levels (i.e. 100–300 mg/kg). The use of hydrogen peroxide as a milk preservative was recommended by FAO in 1957. In the case of the use of H_2O_2 this chemical must be completely destroyed before consumption either by heat treatment or by means of catalase. Previous studies demonstrated that H_2O_2 concentrations lower than 100 mg/kg had no significant effect on the bacterial growth and development of acidity. Hydrogen peroxide may be used as a desirable bactericide in milk and has been suggested as a means of improving milk quality in developing countries. However, milk and milk products are biochemically unstable, i.e. they deteriorate very quickly. Before now, very little work has been done on the use of H_2O_2 as a milk preservative in Bangladesh and no work has been done to monitor the effect of H_2O_2 on dairy products compared to other tropical countries. One exceptional which is found on their experiments is that they do not dilute H_2O_2 before addition to the milk. On this regards under Bangladesh perspective use of hydrogen peroxide (H_2O_2) may be fruitful for preserving milk. Hence the present research work was undertaken to monitor the feasibility of using hydrogen peroxide to preserve milk and to investigate the bacterial population of raw and H_2O_2 treated milk.

2. Materials and Methods

The experiment was conducted at Dairy Technology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University (BAU), Mymensingh from August to November, 2017. The whole milk sample was collected from BAU Dairy Farm, and after milking milk was poured from one pail to another to avoid the incorporation of air .Then it was allowed to stand for a while and thereafter sample was taken in the Laboratory for experimental purpose.

2.1. Experimental procedures

2.1.1. Milk preservation

Collected milk samples after thorough mixing was divided into three equal parts. Out of the three parts, one part was kept as raw milk; untreated milk (control) and rest portions were treated with 0.12 H_2O_2 of 10% concentration. Milk samples were kept in glass beaker at room temperature (31^0C) for shelf life study. Hydrogen

peroxide was diluted at the ratio of 1: 2.49 (H_2O_2: Distilled water) before adding to the milk. There were three treatments in first phase of the experiment which are shown below:

a. Milk sample without any preservative (Raw milk- Control)
b. Milk sample with 0.12% hydrogen peroxide added after 20 minutes of milking
c. Milk sample with 0.12% hydrogen peroxide added after 3 hours of milking.

2.2. Parameters studied

2.2.1. Parameters for detecting initial quality of milk

Before adding different level of H_2O_2 in milk sample the initial quality of milk was examined by some physical and chemical tests. Organoleptic test was performed nasally visually to observe flavor, color and texture. Specific gravity test was performed by using Quevenne Lactometer, according to the method described by Aggarwala and Sharma (1961). Fat test was done according to Babcock fat test method as described by Aggarwala and Sahrma (1961), protein test was done by formal titration method (Bennenberg *et al*., 1949), and acidity test was done as per method described by AOAC, (2003). Detail analytical procedure for each of the above mentioned experiment is given in the appendix.

2.2.2. Parameters to measure shelf life of milk

After treating milk with different level of H_2O_2 following tests were conducted for all the milk samples at every one hour interval until spoilage of milk. Color, flavor, texture, COB and acidity tests were conducted for shelf life evaluation.

2.2.3. Counting bacterial population of milk

To know the effect of H_2O_2 on the bacterial growth rate of milk Standard Plate Count (SPC) of the three fresh samples just after collecting of the milk were conducted. After adding H_2O_2, SPC of the samples were also performed. Finally SPC of the individual samples were done after spoiling of the each samples. The procedure of the SPC of milk is given in the appendices of this thesis.

2.3. Statistical analysis

In this experiment, experimental units (e.g. milk,) were completely homogenous. So, the statistical analysis was done by using Completely Randomized Design (CRD). DMRT in one way ANOVA was used to see the significant difference on acidity among the treatments.

3. Results and Discussion

3.1. Shelf life

3.1.1. Initial quality of milk

The initial quality of milk was monitored before preserving with H_2O_2 in the Dairy technology laboratory. For this purpose some physical and chemical parameters were measured which are shown in the following Table 1.

Table 1. Observation of quality of milk before adding the preservatives (H_2O_2).

Parameters studied	Initial quality of milk (Mean±SD)
Fat (g/kg)	43.0±1.7
SNF (g/kg)	79.5±2.1
Protein (g/kg)	35.6±2.5
TS (g/kg)	122.5±1.8
Water (g/kg)	877.5±1.8
Specific gravity	1.028±0.001
Color (% of normal/abnormal)	Normal 100% (Yellowish white)
Flavor (% of normal/abnormal)	Normal 100% (pleasant aromatic)
Texture	Normal 100% (free flowing liquid)
Clot on Boiling test (COB)	(- ve)
Acidity %	0.14±0.009

The result of this experiment agrees with the work of Islam *et al.* (1984); they stated that out of 35 samples from BAU dairy farm the average specific gravity, fat and acidity were 1.031, 4.80% and 0.15%, respectively and

Imran *et al.* (2008) found that the average moisture, total solids, specific gravity, pH, titratable acidity of cow milk were 86.8±5.02%, 13.5±1.22%, 1.04±0.05, 6.76±0.51, 0.14±0.40%

3.1.2. Shelf life evaluation of H_2O_2 treated milk samples

It is mentioned earlier that physical and chemical parameters were studied after every one hour from control and H_2O_2 treated milk samples. The results obtained are presented below:

Organoleptic parameters

i) Flavor: Flavor of hydrogen peroxide treated and control milk samples are presented in Table 2. It is evident that flavor of fresh milk (without H_2O_2), 0.12% H_2O_2 treated milk (after 20 minutes of milking) and 0.12% H_2O_2 treated milk (after 3 hrs of milking) samples were acceptable up to 10, 14 and 15 hours respectively. After that time flavor was becoming slightly sour. These results showed that hydrogen peroxide has effective action for maintaining the natural flavor of milk.

Table 2. Flavor quality observation of control and different level of H_2O_2 treated milk samples up to 15 hours.

Hour	Level of H_2O_2		
	Raw milk	0.12% H_2O_2 treated milk (after 20 mins of milking)	0.12% H_2O_2 treated milk (after 3 hrs of milking)
0	Pleasing	Pleasing	Pleasing
4	Pleasing	Pleasing	Pleasing
6	Pleasing	Pleasing	Pleasing
8	Pleasing	Pleasing	Pleasing
9	Slight sour	Pleasing	Pleasing
10	Sour	Pleasing	Pleasing
11	Sour	Pleasing	Pleasing
12	Sour	Pleasing	Pleasing
13	Sour	Pleasing	Pleasing
14	Bitter	Sour	Slight sour
15	Bitter	Sour	Sour

ii) Color: The color of untreated and H_2O_2 treated milk samples are shown in Table 3. In this experiment it is evident that of fresh milk (without H_2O_2), 0.12% H_2O_2 treated milk (after 20 minutes of milking) and 0.12% H_2O_2 treated milk (after 3 hrs of milking) samples color was normal up to 10, 14 and 15 hours respectively and after which color become bleached. The result of this experiment agrees with the work of Kang *et al.* (1983).

Table 3. Color quality observation of control and H_2O_2 treated milk samples up to 15 hours.

Hour	Level of H_2O_2		
	Raw milk	0.12% H_2O_2 treated milk (after 20 mins of milking)	0.12% H_2O_2 treated milk (after 3 hrs of milking)
0	Y.W.	Y.W.	Y.W.
4	Y.W.	Y.W.	Y.W.
6	Y.W.	Y.W.	Y.W.
8	Y.W.	Y.W.	Y.W.
9	Bleached	Y.W.	Y.W.
10	Bleached	Y.W.	Y.W.
11	Bleached	Y.W.	Y.W.
12	Bleached	Y.W.	Y.W.
13	Bleached	Y.W.	Y.W.
14	Bleached	Y.W.	Y.W.
15	Bleached	Bleached	Bleached

Y.W. = Yellowish White

iii) Texture:

The texture of all milk samples are shown in Table 4. The normal texture of milk is stated as "free flowing liquid". 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 result of deterioration agrees with the findings of Hossain (1989).

Table 4. Texture quality observation of control and H_2O_2 treated milk samples up to 15 hours.

Hour	Level of H_2O_2		
	Raw milk	**0.12% H_2O_2 treated milk (after 20 mins of milking)**	**0.12% H_2O_2 treated milk (after 3 hrs of milking)**
0	Free flowing	Free flowing	Free flowing
4	Free flowing	Free flowing	Free flowing
6	Free flowing	Free flowing	Free flowing
8	Free flowing	Free flowing	Free flowing
9	Slight clotted	Free flowing	Free flowing
10	Clotted	Free flowing	Free flowing
11	Clotted	Free flowing	Free flowing
12	Clotted	Free flowing	Free flowing
13	Clotted	Free flowing	Free flowing
14	Clotted	Clotted	Free flowing
15	Clotted	Clotted	Clotted

iv) Clot-on- Boiling (COB) Test:

The result of COB test is shown in Table 5. In this result it is clear that untreated milk samples clotted earlier than that of H_2O_2 treated milk samples. The result of this study agrees with the findings of Hossain (1989).

Table 5. Positive COB time of control and of H_2O_2 treated milk samples up to 15 hours.

Hours	Raw milk	0.12% H_2O_2 treated milk (after 20 mins of milking)	0.12% H_2O_2 treated milk (after 3 hrs of milking)
0	-	-	-
4	-	-	-
6	_	_	_
8	-	-	-
9	-	-	-
10	+	-	-
11	+	-	-
12	+	-	-
13	+	_	_
14	+	+	-
15	+	+	+

(+)= COB Positive
(-)= COB Negative

3.2. Chemical parameters

3.2.1. Acidity test

The acidity percent of untreated and H_2O_2 treated milk samples are shown in Table 6. The percentage of acidity increases gradually in all milk samples. The increase was rapid in untreated milk and slow in H_2O_2 treated milk samples. Similar types of results were also observed by Hossain, 1989; Kang *et al.* 1983 and Ambadkar *et al.* 1991. It is well known that the acidity in milk is developed due to the breakdown of milk sugar lactose into lactic acid by the fermentative effect of acid producing bacteria.

Table 6. Acidity of control and H_2O_2 treated milk samples up to 15 hour.

Hour	Raw Milk		0.12% H_2O_2 treated milk (after 20 mins of milking)		0.12% H_2O_2 treated milk (after 3hrs of milking)		P value	Level of Sig.
	Mean	SD	Mean	SD	Mean	SD		
0	0.140	0.000	0.140	0.000	0.140	0.000	-	ND
2	0.145	0.005	0.140	0.020	0.145	0.005	0.850	NS
4	0.160	0.010	0.155	0.005	0.145	0.002	0.076	NS
6	0.175	0.005	0.160	0.010	0.150	0.020	0.145	NS
8	0.185^a	0.005	0.170^b	0.002	0.155^c	0.001	0.0001	**
9	0.195^a	0.002	0.180^b	0.005	0.160^c	0.020	0.032	*
10	0.210^a	0.010	0.190^b	0.010	0.165^c	0.005	0.002	**
11	0.245^a	0.010	0.200^b	0.020	0.175^c	0.002	0.002	**
12	0.270^a	0.020	0.215^b	0.005	0.185^c	0.005	0.0001	**
13	0.350^a	0.020	0.225^b	0.005	0.195^c	0.005	0.0001	**
14	0.490^a	0.010	0.230^b	0.020	0.210^c	0.010	0.0001	**
15	0.580^a	0.020	0.245^b	0.002	0.230^c	0.020	0.0001	**

^{a,b,c} Means with different superscripts differ significantly from each other's within the same row
**= significant level at 1% ($p<0.01$)
*= significant level at 5% ($p<0.01$)
NS= Non-significant
ND= No Difference

3.3. Bacterial growth rate of milk samples

In Table 7 bacterial growth rate of control and H_2O_2 treated milk samples at initial and after spoilage of each samples were shown. Initial bacterial population of raw milk was 83×10^3 and was 290×10^5 after its spoilage. 0.12% H_2O_2 treated milk (after 20 mins of milking) contained 79×10^3 bacteria after adding H_2O_2 and its bacterial population after spoiled was 145×10^5. 0.12% H_2O_2 treated milk (after 3hrs of milking) contained about 200×10^3 and 190×10^5 was after spoiled.

Table 7. Bacterial growth rate of control and H_2O_2 treated milk samples at initial and after spoilage of each samples.

Sample	Initial Bacterial population (Mean ± SD)	Bacterial population after spoilage of milk (Mean ± SD)	Comparison (Mean ± SD)
Raw Milk	$83\pm2.00^c \times 10^3$	$290\pm5.00^a \times 10^5$	$2.89\pm0.10^a \times 10^7$
0.12% H_2O_2 treated milk (after 20 mins of milking)	$79\pm5.00^b \times 10^3$	$145\pm2.00^b \times 10^5$	$1.44\pm1.45^b \times 10^7$
H_2O_2 treated milk (after 3hrs of milking)	$200\pm5.00^a \times 10^3$	$190\pm5.00^c \times 10^5$	$1.88\pm0.05^c \times 10^7$
P value	0.0001	0.0001	0.0001
Level of sig.	**	**	**

^{a,b,c} Means with different superscripts differ significantly from each other within the same row
**= significant level at 1% ($p<0.01$)
NS= Non-significant

4. Conclusions

In this experiment, raw milk was taken immediately after milking from BAU dairy farm and tested shelf life and bacterial growth. From COB test it was found that raw milk sample gave positive result at 10th hour but 0.12% H_2O_2 treated sample (after 20 mins of milking) and 0.12% H_2O_2 treated sample(after 3 hrs of milking) gave positive result at 14 and 15th hour respectively. From the result it was observed that using H_2O_2 shelf life of milk can be increased at 0.12% level and this level was selected for the purpose. Significant difference ($p<0.01$) was found among the level of H_2O_2 in acidity development at every observation hour except 0 hour as the initial acidity was same for all treatments. In order to observe the bacterial growth SPC of the three samples was done two times initially and after spoilage of milk. It was found that bacterial growth was less in the H_2O_2 treated milk than the raw milk sample. Finally we can say that as H_2O_2 inhibits the bacterial growth rate it enhance the shelf life of milk.

Conflict of interest
None to declare.

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