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Enhancement of Shelf-Life of Buffalo Milk Using Hydrogen Peroxide

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ABSTRACT

The research was conducted to weigh the shelf-life and microbial properties in response to adding hydrogen peroxide (H_2O_2) and its residues in buffalo milk. For this purpose, raw milk was collected from the Bangladesh Agricultural University Dairy Farm and treated with different proportions (%) of H_2O_2. Organoleptic, chemical, and microbial tests were done to achieve the aim of the work. Incorporation of different levels of hydrogen peroxide can stabilize the physical, chemical, and microbial properties, where 0.16% H_2O_2 shows better results. For residual effect detection of H_2O_2, up to 0.12% shows a lower disappearance time after boiling. The results of the experiment indicate that H_2O_2 can be used as an excellent preservative.

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Introduction

Milk storage stability, such as shelf life, is important because of its susceptibility to rapid microbial growth, causing spoilage and health risks. Refrigeration effectively slows down bacterial growth but may not be effective in some regions. During the collection and transport of milk, alternative methods are crucial to preserve milk quality. Milk as a nutrient-rich product, supports bacterial growth and begins to deteriorate quickly without an appropriate preservation technique (Omer, 2008). In Bangladesh, most milk is produced in rural areas, where a lack of refrigeration facilities, adequate technology for clean milk production, and also poor transport facilities increase the risk of spoilage during delivery to cities (Athar and Tariq, 1991). Research is needed to develop sustainable milk preservation methods for rural areas without refrigeration.

Hydrogen peroxide (H_2O_2) is considered a potential bactericide for enhancing milk quality. For liquid consumption, safe concentrations range from 100–400 mg/kg (Bjorck, 1987). H_2O_2 is destroyed during heat treatment, making it a viable and effective preservative (Venden Berg, 1985). Inclusion of H_2O_2 within 1 hour of milking has been recommended, and studies have shown its high bactericidal efficiency and relative safety (Nambudripad et al., 1952). The lactoperoxidase (LP) system, naturally present in raw milk, can be activated with H_2O_2 to extend milk's shelf life by 8 to 12 hours. This system needs thiocyanate, hydrogen peroxide, and lactoperoxidase to produce antimicrobial compounds. Research shows that H_2O_2, used in small concentrations (e.g., 0.05%), can inhibit bacterial growth without affecting milk proteins, and any residual H_2O_2 decomposes during boiling. H_2O_2 is particularly useful due to its low cost and ability to preserve milk under ambient temperature in regions like Bangladesh, where refrigeration facilities are limited, and milk must be transported over long distances. Adding H_2O_2 can conserve milk quality, reduce post-harvest losses, and support the economy of small-scale dairy farmers. The objectives of this study include evaluating buffalo milk's shelf life enhancement using H_2O_2, its effect on microbial populations, and its residual activity after heat treatment.

Materials and Method

The experiment was conducted on whole milk collected from Bangladesh Agricultural University Dairy Farm from August to September 2019 at the Dairy Microbiology & Biotechnology Laboratory of the Department of Dairy Science, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. Suggestions were given to the milkmen before milking the buffalo cows for maintaining all hygienic measures. Milk was poured from one pail to another immediately after milking to prevent the incorporation of air and allow it for some time. Thereafter, samples were collected Aseptically in the laboratory for further studies. After collecting milk samples milk was monitored without using H_2O_2 in the Dairy Microbiology & Biotechnology Laboratory where some physical and chemical parameters were measured which proved that the experimental samples source was very good (Table 1).

At first, the milk samples were preserved with different concentrations of H_2O_2. to monitor the effectiveness of this chemical as a preservative of milk. For this purpose, 1 part of Hydrogen peroxide was diluted (30%, w/v) with 2 parts of Distilled water before adding to the milk (Source: www.hydrogen2o2.com/35-food-grade-hydrogen-peroxide) to make 10% H_2O_2 solution. Collected milk samples were divided into five distinct parts which are, untreated milk referred as control (a), treated with 10% H_2O_2 (b), 12% H_2O_2 (c), 14% H_2O_2 (d), 16% H_2O_2 (e), respectively to determine the preservative effect of H_2O_2. The chosen H_2O_2 concentrations were based on previous research findings in different countries in the world. After that different types of evaluations like organoleptic, chemical, and microbial tests were performed on those samples. Organoleptic evaluation was performed by using eyes and nose to record flavor, color, and texture. Specific gravity was assayed by using Quevenne Lactometer, (Aggarwala and Sharma, 1961). Babcock fat test method was used for measuring the fat content of the milk samples, protein test was done by formal titration method (Bennenberg et al., 1949; Aggarwala and Sharma, 1961) and acidity test (by titration) was done as per the method described by A.O.A.C. (1984).

Table 1. Observation of the quality of milk before adding the preservatives

Parameters studied	The initial quality of milk
Fat (g/kg)	76.27±2.21
SNF (g/kg)	86.07±1.58
Protein (g/kg)	39.37±0.49
TS (g/kg)	162.33±3.79
Water (g/kg)	837.67±3.79
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 (%)	0.15±0.01
pH	6.66±0.05

*SNF= Solids Not Fat, TS= Total Solids, %=Percentage, g/kg= Gram per kilogram

After treating milk with different levels of H_2O_2, some assessments like color observation, evaluating flavor and texture, clot on Boiling (COB) test, and acidity test were conducted respectively for all four milk samples every two hours initially followed by 1-hour intervals until spoilage of milk. For evaluating microbial properties, the SPC (Standard Plate Count) method and the number of viable bacteria per ml of milk were assessed at each four-hour interval from the preparation of samples until the spoilage of milk. Finally, H_2O_2 treated milk was explored to evaluate the residual effect using a 2% solution of Paraphenylenediamine followed by different combinations of heat to examine whether it can completely decompose the chemical or not.

In this experiment, one-way ANOVA was done by SPSS (IBM 20) to investigate the treatment effects on milk samples at various hour intervals. Tukey's HSD test was also done to compare the treatment means.

Result and Discussion

Organoleptic Parameters

Flavor

The study evident that the flavor of fresh milk (without H_2O_2), 0.10%, 0.12, 0.14%, and 0.16% H_2O_2 treated milk samples were acceptable up to 9, 11, 13, 14, 15 hours respectively. After that time flavor became slightly sour indicating hydrogen peroxide has an effective action for maintaining the natural flavor of milk (Table 2).

Color

For untreated, 0.10%, 0.12%, 0.14%, and 0.16% H_2O_2 treated milk samples, color was normal up 9, 11, 13, 14, and 15 hours, respectively, after which color from yellowish become bleached. Color deterioration was very rapid in untreated milk, followed by 0.10%, 0.12%, 0.14%, and 0.16% H_2O_2 treated milk samples, and deteriorated with increasing storage time of milk samples. H_2O_2 is an effective chemical for milk preservation and the addition of 0.02% H_2O_2 in milk protects from spoilage for up to 12 hours at 20- 300 at room temperature (Kang et al., 1983).

Table 2. Flavor quality observation of control and different levels of H_2O_2 treated milk samples up to 15 hours at 34.4^{0}C

Hour	Level of H_2O_2				
	Control	**0.10%**	**0.12%**	**0.14%**	**0.16%**
0	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
4	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
6	Pleasing	Pleasing	Pleasing	Pleasing	Pleasing
8	Slight sour	Pleasing	Pleasing	Pleasing	Pleasing
9	Sour	Pleasing	Pleasing	Pleasing	Pleasing
10	Sour	Slight sour	Pleasing	Pleasing	Pleasing
11	Sour	Sour	Pleasing	Pleasing	Pleasing
12	Sour	Sour	Slight sour	Pleasing	Pleasing
13	Bitter	Sour	Sour	Slight sour	Pleasing
14	Bitter	Sour	Sour	Sour	Slight sour
15	Off-flavor	Bitter	Sour	Sour	Sour

Table 3. Color quality observation of control and different levels of H2O2 treated milk samples up to 15 hours at 34.4^{0}C

Hour	Level of H2O2				
	Control	**0.10%**	**0.12%**	**0.14%**	**0.16%**
0	Y.W.	Y.W.	Y.W.	Y.W.	Y.W.
4	Y.W.	Y.W.	Y.W.	Y.W.	Y.W.
6	Y.W.	Y.W.	Y.W.	Y.W.	Y.W.
8	Y.W.	Y.W.	Y.W.	Y.W.	Y.W.
9	Bleached	Y.W.	Y.W.	Y.W.	Y.W.
10	Bleached	Y.W.	Y.W.	Y.W.	Y.W.
11	Bleached	Bleached	Y.W.	Y.W.	Y.W.
12	Bleached	Bleached	Y.W.	Y.W.	Y.W.
13	Bleached	Bleached	Bleached	Y.W.	Y.W.
14	Bleached	Bleached	Bleached	Bleached	Y.W.
15	Bleached	Bleached	Bleached	Bleached	Bleached

Y.W. = Yellowish White

Texture

The texture of untreated, 0.10%, 0.12%, 0.14%, and 0.16% H_2O_2 treated milk samples were normal up to 9, 11, 13, 14, and 15 hours of study respectively and then clotting began which resulted in the untreated one first. Spoilage of raw milk was due to normal souring but H_2O_2 treated milks were spoiled by proteolytic and sweet curdling changes, which indicate H_2O_2 was most effective against souring bacteria.

Table 4. Texture quality observation of control and different levels of H_2O_2 treated milk samples up to 19 hours at 34.4^{0}C

Hour	Level of H_2O_2				
	Control	**0.10%**	**0.12%**	**0.14%**	**0.16%**
0	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
4	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
6	Free flowing	Free flowing	Free flowing	Free flowing	Free flowing
8	Slight clotted	Free flowing	Free flowing	Free flowing	Free flowing
9	Clotted	Free flowing	Free flowing	Free flowing	Free flowing
10	Clotted	Slight clotted	Free flowing	Free flowing	Free flowing
11	Clotted	Clotted	Free flowing	Free flowing	Free flowing
12	Clotted	Clotted	Slight clotted	Free flowing	Free flowing
13	Clotted	Clotted	Clotted	Slight clotted	Free flowing
14	Clotted	Clotted	Clotted	Clotted	Slight clotted
15	Clotted	Clotted	Clotted	Clotted	Clotted

*free flowing= Normal state of milk

Table 5. Average positive COB time of control and different levels of H2O2 treated milk samples up to 15 hours at 34.4^{0}C

Hour	Level of H_2O_2				
	Control	**0.10%**	**0.12%**	**0.14%**	**0.16%**
0	–	–	–	–	–
4	–	–	–	–	–
6	–	–	–	–	–
8	–	–	–	–	–
9	+	–	–	–	–
10	+	–	–	–	–
11	+	+	–	–	–
12	+	+	–	–	–
13	+	+	+	–	–
14	+	+	+	+	–
15	+	+	+	+	+

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

Table 6. Average acidity of control and different levels of H_2O_2 treated milk samples up to 15 hours at 34.4^{0}C

Hour	Level of H_2O_2					P Value	Level of sig.
	Raw milk	0.10%	0.12%	0.14%	0.16%		
0	0.147±0.003	0.147±0.003	0.147±0.003	0.147±0.003	0.147±0.003	1.0	NS
4	0.154±0.003	0.150±0.002	0.148±0.003	0.147±0.003	0.147±0.003	0.040	NS
6	0.164^a ±0.002	0.157^b ±0.002	0.152^c ±0.002	0.149^d ±0.001	0.147^e ±0.001	0.0001	**
8	0.172^a ±0.002	0.164^b ±0.002	0.160^c ±0.001	0.157^d ±0.001	0.150^e ±0.001	0.0001	**
9	0.182^a ±0.002	0.170^b ±00.001	0.163^c ±0.001	0.156^d ±0.001	0.153^e ±0.001	0.0001	**
10	0.192^a ±0.002	0.174^b ±0.001	0.167^c ±0.002	0.159^d ±0.001	0.156^e ±0.001	0.0001	**
11	0.218^a ±0.002	0.182^b ±0.001	0.172^c ±0.001	0.164^d ±0.001	0.160^e ±0.002	0.0001	**
12	0.254^a ±0.003	0.196^b ±0.003	0.176^c ±0.002	0.168^d ±0.001	0.164^e ±0.001	0.0001	**
13	0.318 ^{a} ±0.003	0.214 ^{b} ±0.003	0.183 ^{c} ±0.002	0.172 ^{d} ±0.002	0.168 ^{e} ±0.001	0.0001	**
14	0.415 ^{a} ±0.002	0.263 ^{b} ±0.005	0.234 ^{c} ±0.001	0.184 ^{d} ±0.003	0.174 ^{e} ±0.002	0.0001	**
15	0.481 ^{a} ±0.002	0.325 ^{b} ±0.003	0.297 ^{c} ±0.002	0.215 ^{d} ±0.005	0.184 ^{e} ±0.002	0.0001	**

a, b, c Means with different superscripts differ significantly from each other within the same row
** = significant level at 1% ($p<0.01$), *= significant level at 5% ($p<0.01$), NS= Non-significant

Clot-on-Boiling Test

The COB test was positive at 9, 11, 13, 14, and 15 hours for untreated, 0.10%, 0.12%, 0.14%, and 0.16% H_2O_2 treated milk samples, respectively indicating untreated milk samples clotted earlier than that of H_2O_2 treated milk samples due to more acid production in untreated milk samples. COB test results also confirm that H_2O_2 is an effective preservative for milk preservation by inhibiting the growth-producing bacteria (Hossain et al.,1989; El-Safty et al., 1978; Barbas,1995).

Chemical parameters

The study (Table 6) shows that the acidity of untreated milk increased rapidly, whilst milk treated with H_2O_2 grew slower. At 34.4°C, untreated milk lasted for nine hours, but milk treated with 0.16% H_2O_2 had a fifteen-hour shelf life. After the first hour, there were notable differences ($p<0.01$) in the development of acidity across H_2O_2 levels. Lactose fermentation was reduced to lactic acid by H_2O_2, which inhibited bacteria that produce acid (Park and Pack,1977, Siegenhalar, 1976, Hossain et al., 1989; Gupta et al., 1986).

The present study indicated that the bacterial growth rate is very high in the raw milk ($50.667x10^4$) than 0.10%, 0.12%, 0.14%, 0.16% H_2O_2 treated milk were 48 $x10^4$, 49.333 $x10^4$, 48.667 $x10^4$ and 51 $x10^4$ respectively. So, it can be said that H_2O_2 inhibits the bacterial growth of milk.

Residual effect of milk samples

The experiment shows that H_2O_2 treated samples started to boil within 3 minutes and residual effect was observed in each 1minute interval which shows the residual effect disappeared after 5, 7, 9, and 11min for 0.10%, 0.12%, 0.14%, and 16%, respectively (Table 8).

Table 7. Effect of different levels of treatments at different times on the mean value of microbial count with their standard error

Time of observation	Control/blank sample ($\times10^4$cfu/ml)	0.10% ($\times10^4$cfu/ml)	0.12% ($\times10^4$ cfu/ml)	0.14% ($\times10^4$cfu/ml)	0.16% ($\times10^4$cfu/ml)	p-value
0 h	50.667±1.453	48.000±2.516	49.333±1.764	48.667±2.603	51.000±2.082	0.830
4 h	119.667^{a}±1.764	97.000^{b}±4.509	95.000^{b}±4.163	92.333^{b}±3.844	93.667^{b}±2.404	0.001
7 h	181.333^{a}±1.856	149.000^{b}±4.163	137.333^{c}±1.453	128.667^{cd}±0.882	120.667^{d}±1.202	<0.001
9 h	250.000^{a}±4.041	190.000^{b}±1.732	175.000^{c}±1.155	169.000^{cd}±1.155	160.333^{d}±1.453	<0.001
11 h	n. d.	251.667^{a}±3.528	204.000^{b}± 2.646	190.00^{bc}±4.726	177.667^{c}±0.882	<0.001
13 h	n. d.	n. d.	252.333^{a}±3.528	218.333^{b}±1.202	197.667^{c}± 2.906	<0.001
14 h	n. d.	n. d.	n. d.	253.333^{a}±3.756	219.667^{b}±2.028	0.001
15 h	n. d.	n. d.	n. d.	n. d.	260.000±7.000	–

^{abcd}Mean values in a row with uncommon superscript letters differed significantly. n. d. indicates that not detected due to spoilage of the sample

Table 8. Disappearance time of H_2O_2 from the buffalo milk

Level of H_2O_2	Disappeared time of H_2O_2 after boiling (Minute)
0.10%	2
0.12%	4
0.14%	6
0.16%	9

Conclusion

The results indicated a significant reduction in the progress of the acidity of milk samples treated with H_2O_2 at certain concentrations. A significant alteration was observed among the level of H_2O_2 in acidity development at each observation point except 0 hours. From the results, it was observed that the use of H_2O_2 effectively enhance the shelf life of buffalo milk. In the case of Bacterial count, the bacterial population was less in the H_2O_2 treated milk than in the raw milk sample. The required times of H_20_2 disappeared after sometime boiling.

Conflict

The authors declare no conflict of interest.

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