



## Shelf life of raw and reconstituted milk at room and refrigeration temperature

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### ABSTRACT

The experiment was conducted to evaluate the shelf-life of raw and reconstituted milk at room and refrigerated condition during summer season. Room and refrigeration temperature was 27-29°C and 5-6°C respectively. Each portion of raw and reconstituted milk was divided into two parts and then the samples were stored at room and refrigeration temperature until spoilage. Each experiment was repeated three times. The parameters used to monitor the keeping quality of milk were acidity and clot-on-boiling test. Specific gravity of raw and reconstituted milk was  $1.029 \pm 0.001$  and  $1.032 \pm 0.003$  respectively. COB test was negative for both samples. From the chemical analysis, it was observed that the fat, SNF, TS, lactose, protein, ash and water content were  $41.0 \pm 2.0$ ,  $83.4 \pm 0.15$ ,  $124.4 \pm 1.96$ ,  $48.5 \pm 0.47$ ,  $35.9 \pm 0.2$ ,  $6.8 \pm 0.26$ ,  $875.6 \pm 1.96$  g/kg respectively for raw milk and  $36.3 \pm 0.58$ ,  $100.9 \pm 0.76$ ,  $137.5 \pm 0.5$ ,  $53.7 \pm 0.44$ ,  $36.2 \pm 0.72$ ,  $7.3 \pm 0.21$ ,  $862.5 \pm 0.5$  g/kg respectively for reconstituted milk. Acidity percentage was  $0.142 \pm 0.006$  for raw milk and  $0.168 \pm 0.003$  for reconstituted milk. It was found that at room temperature raw and reconstituted milk were acceptable up to  $9.50 \pm 0.707$  and  $12.00 \pm 1.414$  hours respectively. On the other hand at refrigeration temperature raw and reconstituted milk were acceptable up to  $12.67 \pm 1.155$  and  $23.67 \pm 0.578$  days respectively.

### INTRODUCTION

Milk has been a part of the human diet for thousands of years. It consists of a large number of substances, including protein, fat, lactose, water and minerals, and is often regarded as a complete food as it is capable of sustaining life on its own. Some factors that influence whole milk powder flavor and shelf-life include initial milk quality, processing variables, air quality, moisture content, packaging, oxygen exposure, addition of antioxidants, light exposure and storage temperature (Hall and Lingnert, 1984; Baldwin and Ackland, 1991; Nielsen et al., 1997). Milk and milk products serve as excellent media for the growth and multiplication of microorganisms and thus highly perishable. The quality deterioration of milk not only affects the flavour and nutrition of milk, but also affects the keeping quality.

In the rural areas of our country, there are difficulties or lacking of preservation techniques of raw milk. Most of the farmers or vendors are illiterate; they do not know how to keep or to preserve milk. The keeping quality of milk is

important for the dairy industry both in developed and under developed countries where it is needed to increase the flexibility of processing. Cooling raw milk to 4°C gives it a good keeping quality of at least several days. It is important that milk after having been drawn is cooled rapidly, subsequent transport at temperatures of not more than 4°C is necessary to avoid the increased growth rate of psychrotrophic bacteria. After delivery, the milk should be cooled again to 4°C (Stadhouders, 1982).

Sufficient amount of milk must be available to make it economically viable to cool milk to low temperatures by refrigeration on the farm. When small amount of milk are produced, the cooling of milk should be centralized in collection centers. To control undesirable bacterial development and to maintain high bacteriological standards, the milk should be cooled as quickly as possible after milking, preferably within two hours. If the milk has not been properly cooled shortly after milking, a large variety of microorganisms will start to reproduce (Berg, 1988).

Due to shortage of liquid milk, it is a common practice to produce reconstituted milk which is prepared by mixing whole milk powder with fresh clean water. The manufacturing company gives instruction about what amount of powder milk to be mixed with water to make one liter of liquid milk. Infant powder milk is also reconstituted before giving to baby. Sometimes reconstituted milk is used for the production of different types of dairy products like dahi, cheese, sweetmeats etc. So, reconstituted milk is a part of our daily life. Although there have information about the shelf-life of whole milk under various conditions but no information is available about the shelf-life of reconstituted milk.

From the above discussion it is very much clear that although milk is excellent food item but its nutritive value can be deteriorated if it is not preserved scientifically after milking. Although various scientific preservation techniques are used for milk preservation, but we have very shallow knowledge about how many days we can keep milk in refrigerator in healthy condition. Moreover, shelf-life of milk at room temperature under our climatic condition needs to investigate. Similarly, we do not have adequate information about the shelf-life of raw milk and reconstituted milk at room temperature and refrigerated temperature. Hence the present research has undertaken to know the keeping quality of raw and reconstituted milk under room and refrigeration temperature.

## MATERIALS AND METHODS

### Sources of milk

Whole milk was collected from Bangladesh Agricultural University Dairy Farm with taking the hygienic measure. After milking, the milk was poured from one pail to another. To avoid the incorporation of the air, it was allowed to stand for a while and thereafter sample was taken in the laboratory for experimental purpose. Powdered milk (DANO full cream milk powder) was collected from K. R. Market, BAU campus for preparation of reconstituted milk. It was prepared according to the instruction given on the level of powdered milk. 140 g of powdered milk was dissolved into 1000 ml of clean and fresh water to make the volume of 1L of reconstituted milk.

### Experimental procedure

Raw milk was divided into two equal portions, one portion was kept into room temperature and other portion was kept into refrigerator. Similarly reconstituted milk was divided into two equal portions, one was kept into room temperature and other was kept into refrigerator at 5-6°C. So, finally there were four types of milk samples for the experiment such as raw milk at room temperature, raw milk at refrigerated temperature, reconstituted milk at room temperature and reconstituted milk at refrigerated temperature.

### Analysis of milk sample

The samples were analyzed in the Dairy Technology Laboratory to observe the initial quality of milk samples which were used for measuring storage life. The milk samples were tested for physical and chemical parameters.

### Storage of milk samples

Storage life of both raw and reconstituted milk at room and refrigerated temperature were measured with the help of i) Acidity test and ii) Clot-on-boiling (COB) test.

Table 1  
Tests used for evaluation of initial quality and storage life of milk.

Test	Method used
Specific gravity	Using Quevenne's Lactometer
Total solids (TS) content (%)	Oven drying method
Fat content (%)	Babcock method
Protein content (%)	Formal titration method (A.O.A.C. 2003)
Ash content (%)	Incineration method (A.O.A.C. 2003)
Lactose content (%)	Indirect method (calculation)
Acidity (%)	Titration method (A.O.A.C. 2003)
Clot-on-boiling method	Application of heat (Aggarwala and Sharma, 1961)

## Statistical analysis

In this experiment, experimental materials were completely homogenous. So, the statistical analysis was done by using Completely Randomized Design (CRD) as per Steel and Torrio (1980).

## RESULTS AND DISCUSSION

### Initial quality of milk

#### Physical parameters

Out of various physical parameters, only specific gravity, flavour, colour, texture, taste, temperature and clot on boiling test of milk sample were monitored (Table 2).

#### Flavour

The flavour of all milk samples was normal. All samples showed pleasing flavour and this flavor was mild aromatic (Table 2). The flavor of milk agrees with the report of Judkins and Keener (1960). It has been shown that the pleasing flavour of milk may be correlated with high lactose and relatively low chloride content. Islam et al. (1984) reported that flavour of milk produced hygienically was normal. The flavour of all milk samples collected from B.A.U. Dairy Farm was normal and which agrees with the findings of Biswas et al. (1997) and Saha et al. (1998).

#### Colour

Colour of raw milk samples was yellowish white and reconstituted milk samples was opaque white. No abnormality in colour as detected (Table 2). Lampart (1970) stated that the colour of milk varies upon fat, solids-not-fat (SNF) and the size of the fat globule. The result of the present study, agrees with the work of Islam et al. (1984) who observed that the colour of cow's milk was yellowish white. In this experiment, normal colour of milk samples indicated that no fat had been removed or fat percentage in milk was not too low before starting the experiment.

### Texture

The texture of all milk samples was normal (Table 2). Normal milk texture is designated as free flowing liquid. Its viscosity is higher than water. Acidity development can change the texture of milk. So the results of present study indicated that all milk samples were fresh and no fat had been taken out from the milk. Saha et al. (1998) and Biswas et al. (1997) found similar types of results while working with the fluid milk of BAU Dairy Farm.

### Taste

The taste of all milk samples was slightly sweet which is shown in the Table 2. The result of present study agrees with the findings of Judkins and Keener (1960) and Islam et al. (1984). Judkins and Keener (1960) reported that sweet taste comes from the lactose. Saha et al. (1998) and Biswas et al. (1997) also reported that the normal taste of milk was slightly sweet.

### Specific gravity

The observed average specific gravity of raw milk samples was  $1.029 \pm 0.001$  and reconstituted milk samples was  $1.032 \pm 0.003$  (Table 2). This specific gravity was within the normal range of specific gravity of milk. Islam et al. (1984) found that the average specific gravity of milk of BAU Dairy Farm was 1.031. Quadir (1996) observed that the specific gravity of milk of primary co-operative societies (Milk vita) was 1.027. Similar type of specific gravity of milk of BAU Dairy Farm was obtained by Biswas et al. (1997) and Saha et al. (1998).

### Clot-on-boiling (COB) Test

The results of acidity tests were confirmed by COB test. The COB test showed negative results (Table 2). Biswas et al. (1997) and Saha et al. (1998) found that the COB tests were negative and indicated that there was no developed acidity and the quality of the milk sample were good. The findings of the present experiment were also similar to the report of Khan et al. (1998).

**Table 2**  
Physical parameters of milk samples collected during experimental period.

Physical parameters	Raw milk
Colour	Yellowish white (100%)
Flavour	Normal flavor (100%)
Texture	Normal free flowing liquid (100%)
Taste	Slightly sweet (100%)
Specific gravity	1.029 ± 0.001
Milk temperature	84 ± 2 <sup>0</sup> F
COB test	(-ve)

**Chemical parameters**

**Fat content**

The fat content of raw milk samples was 41.0 ± 2.0 g/kg and reconstituted milk samples was 36.3 ± 0.58 g/kg which is shown in Table 3. Biswas et al. (1997) reported that the average fat content of milk samples collected from BAU Dairy Farm was 37.0 ± 1.0 g/kg. Saha et al. (1998) stated that the average fat content of milk collected from BAU Dairy Farm was 43.0 g/kg.

**Solids-not-fat (SNF) content**

The SNF content of raw milk samples was 83.4 ± 0.15 g/kg and reconstituted milk samples was 100.9 ± 0.76 g/kg which is shown in the Table 3. Yadav and sarawat (1982) in an experiment found that SNF content varied between 63.9 and 88.6 g/kg which is in agreement with the findings of this experiment. In this experiment the SNF content of reconstituted milk samples was higher than the minimum SNF content of milk.

**Total solids (TS) content**

The TS content of raw milk samples was 124.4 ± 1.96 g/kg and reconstituted milk samples was 137.5 ± 0.5 g/kg which is shown in Table 3. Biswas et al. (1997) and Saha et al. (1998) found that the total solids content of the milk of BAU Dairy Farm were 114.2 and 122.5 g/kg respectively.

**Protein**

The protein content of raw milk samples was 35.9 ± 0.2 g/kg and reconstituted milk samples was 36.3 ± 0.17 g/kg which is shown in Table 3. Overman et al. (1953) reported that the average protein of cow's milk was 35.2 g/kg. So their results support the findings of present experiment.

**Lactose**

The Lactose content of raw milk samples was 48.5 ± 0.47 g/kg and reconstituted milk samples was 53.7 ± 0.44 g/kg which is shown in the Table 3. Jenness and Patton (1959) found that generally cow's milk contains 48.0 g/kg lactose.

**Ash**

The ash content of raw milk and reconstituted milk samples was 6.8 ± 0.26 g/kg and 7.3 ± 0.21 g/kg respectively which is shown in Table 3.

**Water content**

The water content of raw milk and reconstituted milk samples was 875.6 ± 1.96 g/kg and 862.5 ± 0.5 g/kg respectively which is shown in Table 3. Biswas et al. (1997) found that the water content of the milk of BAU Dairy Farm was 885.0 g/kg.

**Acidity test**

The acidity of raw milk and reconstituted milk samples was 0.142 ± 0.006 and 0.168 ± 0.003 percent respectively which is shown in Table 3. Similar type of acidity was reported by Biswas et al. (1997) for BAU Dairy Farm milk. Nakae et al. (1978) observed that initial acidity of milk was within the range of 0.13 to 0.15 percent. Similar types of result (0.15%) were reported by Kulkarni et al. (1981) and Dehury et al. (1977). Islam et al. (1984) reported that vendors adulterated milk with water and addition of water decreases the acidity percent of milk. In present study of our experiment, acidity results of raw milk samples collected from BAU Dairy Farm indicated that there was no developed acidity in milk samples and quality was good. On the other hand, acidity of reconstituted milk samples was slightly higher

due to addition of minerals during manufacturing of powdered milk.

Table 3  
Chemical parameters of milk samples collected during experimental period.

Chemical parameters	Raw milk	Reconstituted milk
	Mean $\pm$ SD	Mean $\pm$ SD
Fat (g/kg)	41.0 $\pm$ 2.0	36.3 $\pm$ 0.58
SNF (g/kg)	83.4 $\pm$ 0.15	100.9 $\pm$ 0.76
TS (g/kg)	124.4 $\pm$ 1.96	137.5 $\pm$ 0.5
Lactose (g/kg)	48.5 $\pm$ 0.47	53.7 $\pm$ 0.44
Protein (g/kg)	35.9 $\pm$ 0.2	36.2 $\pm$ 0.72
Ash (g/kg)	6.8 $\pm$ 0.26	7.3 $\pm$ 0.21
Water (g/kg)	875.6 $\pm$ 1.96	862.5 $\pm$ 0.5
% of Acidity	0.142 $\pm$ 0.006	0.168 $\pm$ 0.003

### Shelf-life of milk at room and refrigeration temperature

#### Room temperature (27-29°C)

During summer season raw milk spoiled after 9.50  $\pm$  0.707 hours at room temperature but reconstituted milk spoiled after 12.00 $\pm$ 1.414 hours. Statistical analysis showed that there was significant difference ( $P < 0.05$ ) between the spoilage time of two types of milk at room temperature which is shown in the Table 6. Initial acidity of raw milk and reconstituted milk at the start of the trial were 0.142  $\pm$  0.006 and 0.168  $\pm$  0.003 respectively. Acidity level increased with progress of storage time. Rate of increase of acidity was rapid in raw milk than that of reconstituted milk which is shown in the Table 3. It shows the pattern of change in acidity level. The acidity test was also confirmed by clot-on-boiling test which is shown in the Table 7. During spoilage time the average acidity of raw and reconstituted milk at room temperature was 0.213 $\pm$ 0.003% and 0.283 $\pm$ 0.003% respectively. Practically this spoilage level reached in different times in different samples. For raw milk spoilage time was 9.50 $\pm$ 0.707 hours but for reconstituted milk it was 12.00 $\pm$ 1.414 hours.

The result of this indicates that storage life of reconstituted milk at room temperature was better than raw milk. This was due to the fact that

reconstituted milk was free from bacteria but raw milk contained bacteria. For this reason bacteria present in raw milk multiply rapidly and produce lactic acid in milk quickly than that of reconstituted milk. During summer season temperature was 27- 29°C which is favorable for the growth of microbacterium. For this reason bacterial growth was rapid in summer season and acid production level was also high during summer and milk spoiled rapidly.

#### Refrigeration temperature (5-6°C)

During summer season raw milk spoilage after 12.67 $\pm$ 1.155 days at refrigeration temperature but reconstituted milk spoiled after 23.67 $\pm$ 0.578 days. Statistical analysis showed that there was significant difference between the spoilage time of two types of milk at refrigeration temperature which is shown in the table 6. Initial acidity percentage of raw milk and reconstituted milk at the start of the trial were 0.142  $\pm$  0.006 and 0.168  $\pm$  0.003 respectively. Acidity level increased with progress of storage time. Rate of increase of acidity was rapid in raw milk than that of reconstituted milk which is shown in the Table 4. It shows the pattern of change in acidity level. The acidity test was also confirmed by clot-on-boiling test which is shown in the Table 8. During spoilage time the average acidity of raw and reconstituted milk at refrigeration temperature was 0.215 $\pm$ 0.00% and 0.283 $\pm$ 0.003% respectively.

Practically this spoilage level reached in different times in different samples. For raw milk spoilage time was 12.67 $\pm$ 1.155 days but for reconstituted milk it was 23.67 $\pm$ 0.578 days. The result of this indicates that storage life of reconstituted milk in refrigerated condition was better than raw milk. This was due to the fact that reconstituted milk was free from bacteria but raw milk contained bacteria. For this reason bacteria present in raw milk multiply gradually and produced lactic acid quickly than that of reconstituted milk. The result obtained from this study has similarity with the finding of Stadhouder (1982) observed that cooling raw milk to 4°C gives it a good keeping quality of at least seven days.

Table 4  
Pattern of changes of acidity of raw and reconstituted milk during storage at room temperature (27-29<sup>o</sup>C).

Time (hours)	Raw milk				reconstituted milk			
	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	Mean ± SD	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	Mean ± SD
0	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
1	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
2	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
3	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
4	0.150	0.135	0.145	0.143±0.008	0.165	0.170	0.175	0.170±0.005
5	0.155	0.140	0.160	0.152±0.010	0.175	0.175	0.180	0.177±0.003
6	0.160	0.145	0.165	0.157±0.010	0.195	0.180	0.185	0.187±0.008
7	0.175	0.150	0.165	0.163±0.126	0.210	0.190	0.195	0.198±0.010
8	0.180	0.155	0.170	0.168±0.126	0.235	0.195	0.210	0.213±0.020
9	0.215	0.165	0.175	0.185±0.026	0.240	0.215	0.225	0.227±0.013
10		0.210	0.215	0.213±0.004	0.265	0.225	0.245	0.245±0.020
11					0.280	0.255	0.265	0.267±0.013
12						0.265	0.285	0.275±0.014
13						0.285		0.285

\* Significant at 5% level of probability

Table 5  
Pattern of changes of acidity of raw and reconstituted milk during storage at refrigeration temperature (5-6<sup>o</sup>C).

Time (days)	Raw milk				reconstituted milk			
	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	Mean ± SD	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	Mean ± SD
0	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
1	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
2	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
3	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
4	0.145	0.135	0.145	0.142±0.006	0.165	0.170	0.170	0.168±0.003
5	0.145	0.135	0.150	0.143±0.008	0.165	0.170	0.170	0.168±0.003
6	0.145	0.140	0.150	0.145±0.005	0.170	0.170	0.170	0.170±0.00
7	0.150	0.145	0.150	0.148±0.003	0.170	0.170	0.175	0.172±0.003
8	0.150	0.145	0.165	0.153±0.010	0.170	0.170	0.175	0.172±0.003
9	0.165	0.145	0.170	0.160±0.013	0.185	0.170	0.175	0.177±0.008
10	0.170	0.150	0.175	0.165±0.013	0.190	0.175	0.185	0.183±0.008
11	0.180	0.155	0.180	0.172±0.014	0.190	0.175	0.185	0.183±0.008
12	0.215	0.175	0.215	0.202±0.023	0.190	0.175	0.190	0.185±0.009
13		0.185		0.185	0.200	0.180	0.190	0.190±0.010
14		0.215		0.215	0.200	0.180	0.190	0.190±0.010
15					0.200	0.185	0.210	0.198±0.013
16					0.200	0.185	0.225	0.203±0.020

17	0.215	0.190	0.235	0.203±0.018
18	0.220	0.195	0.245	0.220±0.025
19	0.225	0.225	0.250	0.233±0.014
20	0.235	0.230	0.250	0.238±0.010
21	0.245	0.240	0.260	0.248±0.010
22	0.265	0.255	0.275	0.265±0.010
23	0.280	0.275	0.275	0.277±0.003
24		0.285	0.285	0.285±0.00

\*\* Significant at 1% level of probability

Table 6  
Average spoilage time of raw and reconstituted milk during storage at refrigeration temperature (5-6<sup>0</sup>C).

No. of observation	Spoilage time (days)			
	Room temperature		Refrigeration temperature	
	Raw milk	Reconstituted milk	Raw milk at ref temp	reconstituted milk
Trial I	09	11	12	23
Trial II	10	13	14	24
Trial III	10	12	12	24
Mean ± SD	9.50±0.707	12.00±1.414	12.67 ± 1.155	23.67 ± 0.578

\*\* Significant at 1% level of probability

Table 7  
COB positive test of raw and reconstituted milk during storage at room temperature (27-29<sup>0</sup>C).

Time (hours)	Raw milk			Reconstituted milk		
	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	+	-	-	-	-	-
10		+	+	-	-	-
11				+	-	-
12					-	+
13					+	
Average positive test	9.50±0.707 hours			12.00±1.414 hours		

\* Significant at 5% level of probability

Table 8  
COB positive test of raw and reconstituted milk during storage at refrigeration temperature (5-6<sup>0</sup>C).

Time (days)	Raw milk			Reconstituted milk		
	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial	1 <sup>st</sup> trial	2 <sup>nd</sup> trial	3 <sup>rd</sup> trial
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	-	-	-	-
8	-	-	-	-	-	-
9	-	-	-	-	-	-
10	-	-	-	-	-	-
11	-	-	-	-	-	-
12	+	-	+	-	-	-
13	-	-	-	-	-	-
14	-	+	-	-	-	-
15	-	-	-	-	-	-
16	-	-	-	-	-	-
17	-	-	-	-	-	-
18	-	-	-	-	-	-
19	-	-	-	-	-	-
20	-	-	-	-	-	-
21	-	-	-	-	-	-
22	-	-	-	-	-	-
23	-	-	-	+	-	-
24	-	-	-	-	+	+
Average COB positive test	12.67 ± 1.155 days			23.67 ± 0.578 days		

\*\* Significant at 1% level of probability

Room temperature was within the range of 27-29<sup>0</sup>C and refrigeration temperature was within the range of 5-6<sup>0</sup>C. At room temperature raw milk and reconstituted milk were acceptable up to 9.50 ± 0.707 and 12.00 ± 1.414 hours respectively. On the other hand at refrigerated condition raw and reconstituted milk were acceptable up to 12.67 ± 1.155 days and 23.67 ± 0.578 days respectively.

**CONCLUSION**

Based on the parameters studied it was found that quality of raw and reconstituted milk collected during experimental period was good. Storage life of fresh milk was found less than that of the

reconstituted milk both at room and refrigerated condition in summer season.

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