Freezing Effect as a Post-Harvest Storage Technique: Quality of Buffalo Milk

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ABSTRACT

The current study evaluates the effect of freezing types at −20 ± 1 °C on the density, freezing point, specific gravity, fat, protein, and lactose of buffalo milk. Buffalo milk samples were collected and pooled separately in the morning. Milk samples were transferred immediately to the Dairy Chemistry and Technology Laboratory. Organoleptic tests were done to observe flavour and odour, sediment, and texture. Physico-chemical and milk compositions were analyzed by using an automatic ultrasonic milk analyzer calibrated for buffalo milk. In the case of organoleptic assessment, a slightly flat flavour was observed at 168 h of freezing in the single-stage but in the case of multi-stage of freezing, the slightly flat flavour had been observed at 120 h. The physicochemical parameters such as the density, freezing point, specific gravity and the compositions of fat, protein and lactose content of the milk samples were significantly different (p < 0.05) for both single and multi-stage freezing types. It was noted that the milk’s fat, protein, and lactose levels decreased as freezing times increased for both freezing procedures. In conclusion, the milk samples were frozen at −20 ± 1 °C significantly decreased fat, protein, and lactose content. Samples were frozen, thawed, and then refrozen (multi-stage freezing), the loss of components was noticeably greater than when the samples were frozen once (single freezing).

Keywords: Buffalo milk, freezing time, freezing type, shelf life.

1. INTRODUCTION

Many of the essential nutrients required for the growth and maintenance of the human body are found in milk [1]. Regarding being essential for human nutrition, milk and dairy products are thought to be the best sources of water-soluble vitamins, calcium, essential fatty acids, and proteins with higher biological value [2]. The most excellent resource of nutrition, particularly suited for both prenatal and neonatal infants, has been and remains human milk [3] but also for low-birth-weight newborns [4]. However, infant formulae and human milk are not widely available where animal-derived milk, such as cow and buffalo milk, contribute to fostering the nutritional requirements of infant supplementation and household consumption. Linolenic and conjugated linolenic acids, fat, lactose, casein, amino acids, and calcium are all in significant buffalo milk concentrations [5]. Importantly, the higher concentrations of fatty acids and amino acids in buffalo milk serve to avoid metabolic syndrome, chronic illnesses, hypertension, and Type 2 diabetes [6]. However, most of the milk in our country is marketed in the informal market as a liquid. Consequently, this milk has a short shelf life of typically three to five days [7]. Freezing milk is said to be the greatest method to maintain its nutritional value, prevent spoiling, and extend its shelf life due to its better storability and convenience rather than preservation with chemicals [8]–[10]. Fat separation, protein flocculation, and the development of an off-flavour could all be negatively impacted by freezing, which could also have an impact on the stability attributes of milk. These alterations affect not only the shelf life but also the quantity of macro and micro-nutrients of milk and dairy products. Many people might have a propensity to routinely freeze large quantities of milk, thaw it out, and then refreeze what is left of it. A previous study recommended that milk should be stored in small quantities, which implies single feeding [11]. In this respect, previous studies have been carried out to observe the effect of freezing on the macro and micro-nutrients of human milk [12]–[14], cow milk [15]
and sheep milk [16]. However, the freezing effect on the compositional quality of buffalo has not been revealed yet. Therefore, it is necessary to assess the impact of single and multi-stage freezing in combination with time on the nutritional compositions of buffalo milk.

2. Materials and Methods

2.1. Site of the Experiment

Three non-descriptive buffalo (n = 3) were selected from Bangladesh Agricultural University Dairy Farm (BAUDF) (24°43′46.5″ N, 90°25′22.8″ E), Mymensingh based on parity (all buffaloes were in 3rd parity) and lactation stage (120 ± 10 days). Buffaloes were fed on German grass (Echinochloa crusgalli) in a cut-and-carry system, with a concentrated mixture as a feed supplement. Buffaloes had unlimited access to free, clean drinking water for a full day.

2.2. Collection of Milk Sample

Buffalo milk samples were collected and pooled separately in the morning before feeding from the BAUDF. Six litres of milk samples from three buffaloes (2 litres from each) were collected aseptically and mixed thoroughly in a bucket. After proper mixing, 3 litres of milk samples were immediately cooled in a cool box containing ice packs. Milk samples were transferred immediately to the Dairy Chemistry and Technology Laboratory of Bangladesh Agricultural University. For 0-hour results, milk samples were analyzed in 10 replications and used for both single and multi-stage freezing techniques (Fig. 1).

2.3. Freezing of Milk Sample

Two forms of freezing were applied to the samples: single freezing and multi-stage freezing. To preserve milk samples 50 ml sterile polypropylene centrifuge tubes with labels were used at −20 ± 1 °C in a deep freezer. The following arrangement of four aliquots was used to estimate the milk content for single freezing: 24 h, 72 h, 120 h, and after 168 h. Ten samples of 50 ml were measured at each time as replication, thus covering a total of 40 milk samples. All the analyzed samples were discarded after analysis at each measurement. In the case of multi-stage freezing, 10 milk samples of 50 ml each were analyzed at 24 h, 72 h, 120 h, and 168 h of freezing. For multi-stage freezing, after one analysis, the samples were refrozen for the next measurement, thus covering a total of 10 samples. Before the next analysis, milk samples were thawed for 40 to 50 min by keeping the sample tubes in the water bath at 23 °C and mixed properly by shaking for 30 s (Fig. 1).

2.4. Organoleptic Test/Sensory Evaluation

Five skilled and expert judges were selected for the organoleptic test from the Department of Dairy Science, Bangladesh Agricultural University (BAU). Judges were selected based on their ability to detect the basic organoleptic test. Organoleptic tests were done to observe flavour and odour, sediment and texture. Odour was detected by sniffing; the flavour was detected by sniffing, followed by placing the samples in the mouth to come in contact with the test bud. The texture of the milk sample was observed visually whereas the sediment was observed by filtering the milk.

2.5. Milk Compositions Analysis

An automatic ultrasonic milk analyzer (Lactoscan MCC, SLP 60, V60, Bulgaria), calibrated for buffalo milk, was used to measure the density, freezing point, specific gravity, fat, protein, and lactose in milk samples.

2.6. Statistical Analysis

One-way analysis of variance was carried out to determine any significant differences among the milk composition of the milk samples at different times of freezing in single and multi-stage types by using IBM SPSS 22 software (SPSS Inc., Chicago, IL, USA). The Tukey HSD test was carried out to determine the significant difference between different treatment means. To investigate relationships between the length of freezing and any variation in the macronutrient composition of buffalo milk, the linear model shown below was fitted:

\[ Y = a + Xb \]

where Y denotes a projected change in milk parameter content as the dependent variable, based on the freezing time h, X denotes the vector of the independent variable, a denotes as intercept, and b denotes the regression slope.
3. Results and Discussion

3.1. Sensory Properties of Buffalo Milk

The organoleptic parameters such as flavour, odour and texture showed no significant changes up to 120 h of freezing in both the single-stage and multi-stage stages (Table I).

A slightly flat flavour was observed at 168 h of freezing in the single-stage but in the case of multi-stage of freezing, the slightly flat flavour had been observed at 120 h and 168 h of freezing. According to certain investigations, off-flavour was encouraged by lipid oxidation, lipolysis, and proteolysis during freezing [16], [17]. However, no sediment was noticed in single-stage freezing whereas slight sediment was observed at 120 h and 168 h in multi-stage freezing. The breakdown of milk fat globule membranes during repeated freezing and thawing may result in very little sedimentation. In addition, texture showed no significant changes till 168 h of freezing in both stages. The texture of the milk was free-flowing for both types of freezing.

3.2. Physicochemical Parameters and Milk Compositions of Buffalo Milk

The physicochemical characteristics of milk, including its density, freezing points, and specific gravity, significantly (p < 0.01) varied during freezing times for both single-stage and multi-stage freezing (Table II).

In each stage of freezing, specific gravity increased significantly from 1.031 to 1.055 in single-stage and from 1.031 to 1.052 at 0 to 168 h in multi-stage freezing. On the other hand, density tended to decrease with the freezing times. This increasing trend of specific gravity in both types of freezing may be due to loss in milk fat with freezing times as fat is the lightest part of all milk constituents. The current study's initial specific gravity was 1.033, which is in line with the findings of Mahmood and Usman [18] for buffalo milk. The average specific gravity of buffalo milk was found to be 1.033, as reported by Pece et al. [19]. The density in the multi-stage freezing technique was reduced radically from 22.5 to 20.06 g/cm³. The declining pattern of density in both stages of milk freezing might be influenced by the reduction of total solids along with fat, protein, and lactose with consecutive freezing times. An essential physical and qualitative indicator of milk is its freezing point. It is primarily used to regulate the quality of raw or pasteurized milk to prevent unintentional water adulteration of milk. It is manifested that the freezing point for both storage techniques was in the normal range as the standard freezing point of milk is −0.520 °C to −0.505 °C [20]. The freezing point of milk samples increased with increasing freezing duration. This increasing scenario of freezing point might be for decreasing total solids, milk fat, and proteins [20]. However, in the multi-stage freezing, this increase in freezing time was more noticeable, which could be related to repeated thawing and freezing. A study of when freezing alters the physicochemical characteristics of sheep milk in nature [21] came to a similar conclusion.

It was investigated that the chemical composition of milk, such as milk fat, protein and lactose percentage showed a decreasing trend with the advancement of freezing time in both stages of freezing. However, a comparatively higher declining rate of milk fat, protein and

### Table I: Effects of Freezing Time on Sensory Properties of Buffalo Milk

<table>
<thead>
<tr>
<th>Freezing type</th>
<th>Parameters</th>
<th>0 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-stage</td>
<td>Flavour and odour</td>
<td>Normal</td>
<td>No sediment</td>
<td>Normal</td>
<td>No sediment</td>
<td>Normal</td>
<td>Slightly flat flavour</td>
</tr>
<tr>
<td></td>
<td>Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Texture</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td></td>
</tr>
<tr>
<td>Multi-stage</td>
<td>Flavour and odour</td>
<td>Normal</td>
<td>No sediment</td>
<td>Normal</td>
<td>No sediment</td>
<td>Normal</td>
<td>Slightly flat flavour</td>
</tr>
<tr>
<td></td>
<td>Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Texture</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td>Free-flowing</td>
<td></td>
</tr>
</tbody>
</table>

### Table II: Effects of Freezing Time on Physical Properties and Milk Compositions (Mean ± SE) of Buffalo Milk

<table>
<thead>
<tr>
<th>Freezing type</th>
<th>Parameters</th>
<th>0 h</th>
<th>24 h</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-stage</td>
<td>Density (g/cm³)</td>
<td>22.59 ± 0.04b</td>
<td>21.36 ± 0.18b</td>
<td>21.29 ± 0.02b</td>
<td>20.92 ± 0.45b</td>
<td>20.76 ± 0.29b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Freezing point</td>
<td>0.5149 ± 0.000b</td>
<td>0.4798 ± 0.003b</td>
<td>0.4672 ± 0.002ab</td>
<td>0.4605 ± 0.006d</td>
<td>0.4804 ± 0.002ab</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Specific gravity</td>
<td>1.031 ± 0.00b</td>
<td>1.034 ± 0.00b</td>
<td>1.034 ± 0.00b</td>
<td>1.034 ± 0.00b</td>
<td>1.035 ± 0.00a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fat (%)</td>
<td>9.04 ± 0.01a</td>
<td>9.00 ± 0.12ab</td>
<td>8.49 ± 0.05bc</td>
<td>8.21 ± 0.17c</td>
<td>7.19 ± 0.17d</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Protein (%)</td>
<td>3.91 ± 0.00a</td>
<td>3.81 ± 0.01b</td>
<td>3.68 ± 0.01c</td>
<td>3.59 ± 0.02d</td>
<td>3.5 ± 0.02c</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Lactose (%)</td>
<td>4.22 ± 0.00a</td>
<td>4.08 ± 0.01bc</td>
<td>4.14 ± 0.05ab</td>
<td>3.91 ± 0.04c</td>
<td>3.99 ± 0.02bc</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multi-stage</td>
<td>Density (g/cm³)</td>
<td>22.5 ± 0.04a</td>
<td>21.56 ± 0.51ab</td>
<td>20.34 ± 0.29bc</td>
<td>19.67 ± 0.32c</td>
<td>20.06 ± 0.72bc</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Freezing point</td>
<td>0.5149 ± 0.000a</td>
<td>0.4877 ± 0.005b</td>
<td>0.4695 ± 0.005a</td>
<td>0.4912 ± 0.003b</td>
<td>0.4808 ± 0.001ab</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Specific gravity</td>
<td>1.031 ± 0.00b</td>
<td>1.034 ± 0.00b</td>
<td>1.041 ± 0.00b</td>
<td>1.051 ± 0.00d</td>
<td>1.052 ± 0.00b</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fat (%)</td>
<td>9.04 ± 0.01a</td>
<td>8.83 ± 0.15bc</td>
<td>8.620 ± 0.06ab</td>
<td>7.98 ± 0.03c</td>
<td>6.83 ± 0.11d</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Protein (%)</td>
<td>3.91 ± 0.00a</td>
<td>3.67 ± 0.03bc</td>
<td>3.58 ± 0.01d</td>
<td>3.56 ± 0.02d</td>
<td>3.72 ± 0.02a</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Lactose (%)</td>
<td>4.22 ± 0.00a</td>
<td>4.03 ± 0.04b</td>
<td>3.94 ± 0.03b</td>
<td>4.00 ± 0.05b</td>
<td>3.89 ± 0.03c</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Note: Data for freezing time is presented as mean ± SE, abcd Mean with different superscripts within a row are significantly different (p < 0.05).
Fat globules are subsequently broken down, reducing their size. According to Varricchio [32], the amount of milk fat, protein, and lactose considerably decreased as the freezing duration increased. They observed that the decline in multi-stage frozen samples was greater than that in single frozen samples. Abranches et al. [14] evaluated the effects of freezing and thawing on the fat, protein, and lactose content of continuously infused natural human milk. The freezing process can significantly affect fat globules, increasing the release of lipoproteins and reducing the stability of the lipid phase, according to Tribst et al. [32] and Wendorff and Kalit [23]. Longer freezing durations in the aforementioned study led to lower levels of milk parameters. According to earlier research by Abranches et al. [14] and García-Lara et al. [12], milk components can change physically and chemically when frozen and thawed. The result of the present study also supports the findings of [24] and [25]. In frozen cow’s milk, Moreno and Emata [26] found that the fat (2.97%) and fat (4.07%) dramatically decreased. They observed that the decline in multi-stage freezing (9.04% to 6.83%) at 0 to 168 h. Nevertheless, the multi-stage freezing showed a more noticeable decrease in single-stage freezing (9.04% to 7.19%) and multi-stage freezing (9.04% to 7.97%). In this investigation, milk fat dramatically dropped in single-stage freezing (9.04% to 7.19%) and multi-stage freezing (9.04% to 6.83%) at 0 to 168 h. Nevertheless, the multi-stage freezing showed a more noticeable decrease with freezing duration in Fig. 2.

The results aligned with earlier research that showed reduced milk fat following an 80-day freezing period of goat milk at a temperature between −16 °C and −20 °C [29], and sheep milk at a temperature of −15 °C or −25 °C [16]. When cow milk was chilled to 4 °C, similar outcomes were also noted by Rico et al. [30] and Peter et al. [31]. Repeated freezing and thawing of the fat globule damages the membrane, causing the substrate to be more accessible to the suppressed enzyme activity [32]. Triglycerides in fat globules are subsequently broken down, reducing their content. The milk protein had a consistent decrease after freezing and thawing.

In single freezing, protein content decreased from 3.91% to 3.59% at 0 h to 168 h, consecutively. Similarly, in multi-stage freezing, protein content reduced from 3.91% to 3.72% likewise the single freezing times. The decreasing pattern of protein content was pronounced more for single-stage freezing than multi-stage freezing type (Fig. 3).

Buffalo milk is ideal for making butter, cream, and yoghurt because of its high protein and fat content, which gives it a rich and delicate texture. In particular, the trend towards lower protein concentration is consistent with a prior study on the effects of freezing or refrigeration on the macronutrients in human milk reported by Păduraru et al. [33]. The decrease in protein has been attributed to several factors. Casein micelles are frequently less stable during cold storage because of physical aggregation and a decrease in the hydrophobic contacts between casein molecules inside the micellar structure [34]. The lactose content of milk samples in both freezing systems decreased significantly (p < 0.01), and the decreasing trends for each technique were almost similar (Fig. 4). The lactose content in the single-stage decreased by 0.23% from 0 h to 168 h and in the multi-stage by 0.33%.

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**Fig. 2.** Relationship between duration of time and fat content with freezing (SS: Single-stage, MS: Multi-stage).

**Fig. 3.** Relationship between duration of time and protein content with freezing types (SS: Single-stage, MS: Multi-stage).

**Fig. 4.** Relationship between duration of time and lactose content with freezing types (SS: Single-stage, MS: Multi-stage).
from 0 h to 168 h. According to Ali et al. [35], the lactose content of buffalo milk from Trishal Thana’s Senbari bazaar in the Mymensingh district was 4.4%. The average lactose concentration of cow milk was found to be 4.31% and 4.38%, respectively, by Imran et al. 2008 [36].

4. CONCLUSION

In our study, we observed the freezing effects on the physicochemical and nutritional compositions of buffalo milk. Though freezing is the most widely used storage technique, it influences the milk compositions. We found that milk compositions such as fat, protein, and lactose decreased remarkably along with diminished physicochemical qualities over the freezing time. However, the milk component loss was more pronounced in multi-stage freezing than in single freezing.

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AUTHORS’ CONTRIBUTION


CONFLICT OF INTEREST

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

REFERENCES


