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Effects of freezing as a post-harvest storage technique on quality of Friesian crossbred cattle milk

Lilian Mutheu Musembei*, Rawlynce Cheruiyot Bett, Charles Karuku Gachuiri, Benjamin Kyalo Mbondo and Felix Matura Kibegwa

Department of Animal Production, Faculty of Veterinary Medicine, University of Nairobi, Kenya.

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Twenty-five raw milk samples from Friesian crossbred dairy cows were analyzed for milk fat, protein and lactose using an ultrasonic milk analyzer. The samples were then subjected to two different freezing protocols; single freezing and multiple freezing, after which parameters were reanalyzed after freezing and thawing at different freezing times (24, 48, and 72 h). Paired t-test was used to compare the effect of freezing type while the differences in milk constituents with freezing time were analyzed using ANOVA. Linear regression analysis was also performed to study correlations between freezing duration and any change in cattle milk's macronutrient content. The results indicated that milk fat, protein and lactose content decreased significantly with freezing time. However, the decrease was more in multiple frozen samples than single frozen samples. The most decreased macronutrients were lactose (14.1%) in single freezing and fat (25.5%) in multiple freezing. Analysis of the interaction between freezing type and freezing time showed that freezing time significantly affected all the parameters while freezing type (p=0.03) and its interaction with freezing time (p=0.02) affected only the fat content. In conclusion, it should be noted that cattle milk samples frozen at -20°C leads to a significant decrease in fat, protein, and lactose content. The loss of constituents was much more pronounced when samples were frozen, thawed, and refrozen (multiple freezing) than when samples were thawed only once (single freezing).

Key words: Single freezing, multiple freezing, macronutrients, dairy cattle.

INTRODUCTION

Milk and other animal-source foods are concentrated dietary sources of macro- and micronutrients such as proteins, carbohydrate, calcium, phosphorus and vitamin B2, B12. Milk is an incredibly important form of animal-source food since it is intended for nurturing the young, a population group at high risk for nutritional deficiencies. Worldwide, nearly 229.2 million children below five years were affected by malnutrition by 2019, with 144 million stunted, 47 million wasted, and 38.2 million overweight (UNICEF/WHO/World Bank Group, 2020). In Kenya, under-nutrition affects nearly one third of children (KNBS, 2015). This under-nutrition increases disease risk, restricts cognitive development, and impedes human capital accumulation. Human milk has been and still is

*Corresponding author. E-mail: linnetmusembei@gmail.com, Tel: +254719543831.

the best source of nutrition that is uniquely suited not only for term and preterm infants (Victora et al., 2016) but also for very low birth weight (VLBW) infants (Arslanoglu et al., 2019), conferring both short- and long-term health benefits. It is recommended that the delivery of this milk to the infant be through breastfeeding. However, sometimes breastfeeding may not be possible due to mother and infant contraindications (Szajewska et al., 2016).

Usually, in such cases, in developed countries, human milk bank for hospitalized babies has been considered as the first choice. However, the emergence of immune debilitating diseases like HIV/AIDS and the development of infant formulas have highly affected the human milk bank's popularity (Leaf and Winterson, 2009). Conversely, in developing countries, where milk banks and infant formulas are not easily accessible, one way to foster infants' nutritional status is by increasing their consumption of livestock-derived foods (LDF), especially milk. Milk produced by domestic animals is not only consumed by infants but also by other age groups.

In Kenya, dairy cattle produce more than 56% of the country's overall milk production (Odero-Waitituh, 2017). This makes cattle milk the most common and readily available milk source for infant supplementation and household consumption (Muriuki, 2011). Over 70% of this milk is marketed raw through informal markets (Muriuki, 2011). Therefore, this milk has a shortened shelf life, usually between three to five days (Ajmal et al., 2018). To preserve the nutritional value, avoid spoilage and increase this milk's shelf life, players along the milk value chain have considered freezing as a good solution due to its greater storability and convenience (Pollack, 2001). However, many consumers tend to freeze the milk in bulk, thaw, and then refreeze the remaining aliquot of milk. Bulk milk storage contrasts with previous studies' recommendations that milk should be stored in small amounts that are consumable within one feeding (Alinovi et al., 2020). Further, many previous studies on effect of freezing on quality have been on human milk (Abranches et al., 2014; García-Lara et al., 2012), with very few studies on the effect of freezing on cattle milk's macronutrients content (Weese et al., 1969). As such, there is a need to assess the impact of freezing and refreezing thawed milk, as a post-harvest storage technique, on cattle milk's nutritional composition. Therefore, this study seeks to evaluate the effects of freezing (type and time) on the cattle milk composition.

MATERIALS AND METHODS

Experimental animals

The experiment was approved by the Faculty of Veterinary Medicine, University of Nairobi's Institutional Animal Care and Use Committee (IACUC), Reference number: FVM BAUEC/2020/268. The experiment was conducted at the University of Nairobi Veterinary farm. The farm lies between latitudes 1° 14' S and 33° 4' S and longitudes 36° 42' E and 36° 3' E. Twenty-five Friesian crossbred dairy animals (350 ±50 kg body weight) were randomly selected from the farm herd for use in this study.

Milk sampling

The samples were collected in morning milking (6 am) prior to feeding. Milk samples were collected aseptically and placed in labeled sterile 50 ml polypropylene centrifuge tubes. Samples were immediately placed in ice cooled container and transported to the laboratory for analysis. Twenty-five 50 ml samples of fresh milk from each cow were analyzed for milk composition parameters on the day of collection, 0 h, then divided into four portions of 10 ml aliquots. The samples were then allocated either into single freezing or multiple freezing protocol (freezing type). For single freezing, milk composition was determined on three aliquots of each sample in the following order: (i) after 24 h (aliquot 1), (ii) after 48 h (aliquot 2), and (iii) after 72 h (aliquot 3). All aliquots were discarded after taking measurements at each time point. Determination of milk composition for the multiple freezing was done on the fourth aliquot for each sample, after 24, 48 and 72 h. Following each of the measurements, in the latter experiment, the samples were refrozen and then thawed for the next measurements before being discarded after analysis at 72 h. All samples were frozen at -20 ± 1°C (Kamelska et al., 2012; Pietrzak-Fiećko and Kamelska-Sadowska, 2020) in a DW-40W380 Haier Deep Freezer (Haier Medical Laboratory Products. Co., Ltd. Qingado, China). Prior to the analysis, samples were left to thaw at room temperature for 45-60 min and homogenized by shaking for 30 s (Figure 1).

Milk parameter measurement and Statistical analysis

Milk fat, protein, and lactose, for all the samples, were determined using an automatic ultrasonic milk analyzer (Lactoscan MCC, SLP 60, V60), calibrated for cattle milk. Statistical analysis was performed on the various milk nutritional parameters using SPSS version 25 SPSS Inc, Chicago, Ill). Paired t-test was used to compare macronutrient concentrations of single freezing and multiple freezing experiments. In addition, analysis of variance (ANOVA) was used to assess for differences in milk constituents from fresh milk and over previously frozen milk at different times. The means were then compared using the Bonferroni posthoc test. Variables were expressed as percent mean ± SE. A p-value of $p \leq 0.05$ was considered significant. The linear model described below was fitted to study correlations between freezing duration and any change in cattle milk's macronutrient content:

$$Y = a + X\beta$$

where Y is the predicted change in individual milk parameter content, the dependent variable, depending on the duration of freezing (hours), X, the vector of the independent variable, a is the intercept, a constant, and β is the regression slope

RESULTS

The milk fat, protein, and lactose contents significantly decreased with an increase in duration of freezing, from 0 to 72 h, for single freezing ($p<0.05$) and multiple freezing ($p<0.001$) (Table 1). Additionally, the decline in all the parameters had a significant correlation with the freezing duration (Figure 2). When samples for single and multiple freezing experiments were compared at different times, it

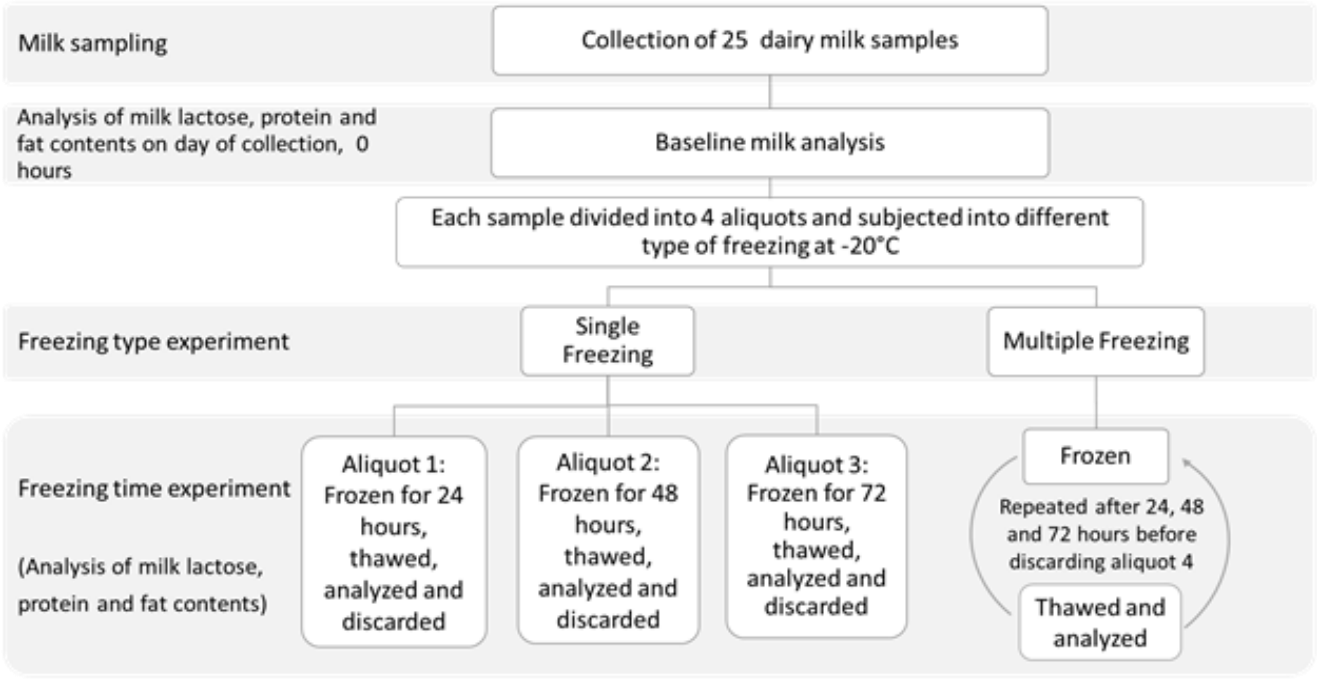

Figure 1. The scheme of sample analysis.

Table 1. Effects of type of freezing on milk composition of Friesian crossbred dairy cattle.

Freezing type	Parameter	Freezing time (hours)				P-value
		0	24	48	72	
Single	Fat (%)	3.81±0.12^{a}	3.59±0.10^{a}	3.4±0.11^{a}	3.39±0.10^{a}	0.042
	Protein (%)	2.03±0.04^{b}	1.92±0.05^{ab}	1.83±0.05^{a}	1.81±0.05^{a}	0.005
	Lactose (%)	2.13±0.06^{b}	1.96±0.05^{ab}	1.85±0.06^{a}	1.83±0.06^{a}	0.002
Multiple	Fat (%)	3.81±0.12^{c}	3.59±0.10^{bc}	3.29±0.10^{b}	2.84±0.13^{a}	<0.001
	Protein (%)	2.03±0.04^{b}	1.92±0.05^{ab}	1.77±0.05^{a}	1.76±0.05^{a}	<0.001
	Lactose (%)	2.13±0.06^{b}	1.96±0.05^{ab}	1.77±0.07^{a}	1.76±0.06^{a}	<0.001

Data for freezing time is presented as mean ± SE, abMean with different superscripts within a row are significantly different (P<0.05)

was observed that most milk parameters were lowest for multiple freezing at 48 and 72 h. Assessment of the magnitude of parameter decline between the fresh milk and the milk parameters at 72 h revealed that all the parameters decreased with a higher magnitude for multiple freezing than single freezing. Specifically, lactose, 14.1%, and fat content, 25.5%, were the parameters that decreased the most during both single and multiple freezing. Analysis of the interaction between freezing type and freezing time showed that freezing time had a significant effect on all the parameters while freezing type (p=0.03) and its interaction with freezing time (p=0.02) affected only the fat content (Table 2).

DISCUSSION

Milk is of nutritional value to both young and adults because of its complex constituent's mixture of fats, proteins, carbohydrates, minerals, vitamins, and other miscellaneous constituents dispersed in water (chloride, sodium, and urea). Although milk composition is unique for each species, the presence of the same nutrients renders milk from different species substitutable, e.g., bovine milk has been used to feed infants where human milk is not available. The milk water content is approximately 88% for both bovine and human milk while milk fat, lactose and protein is content is 3.9 and 4.1%,

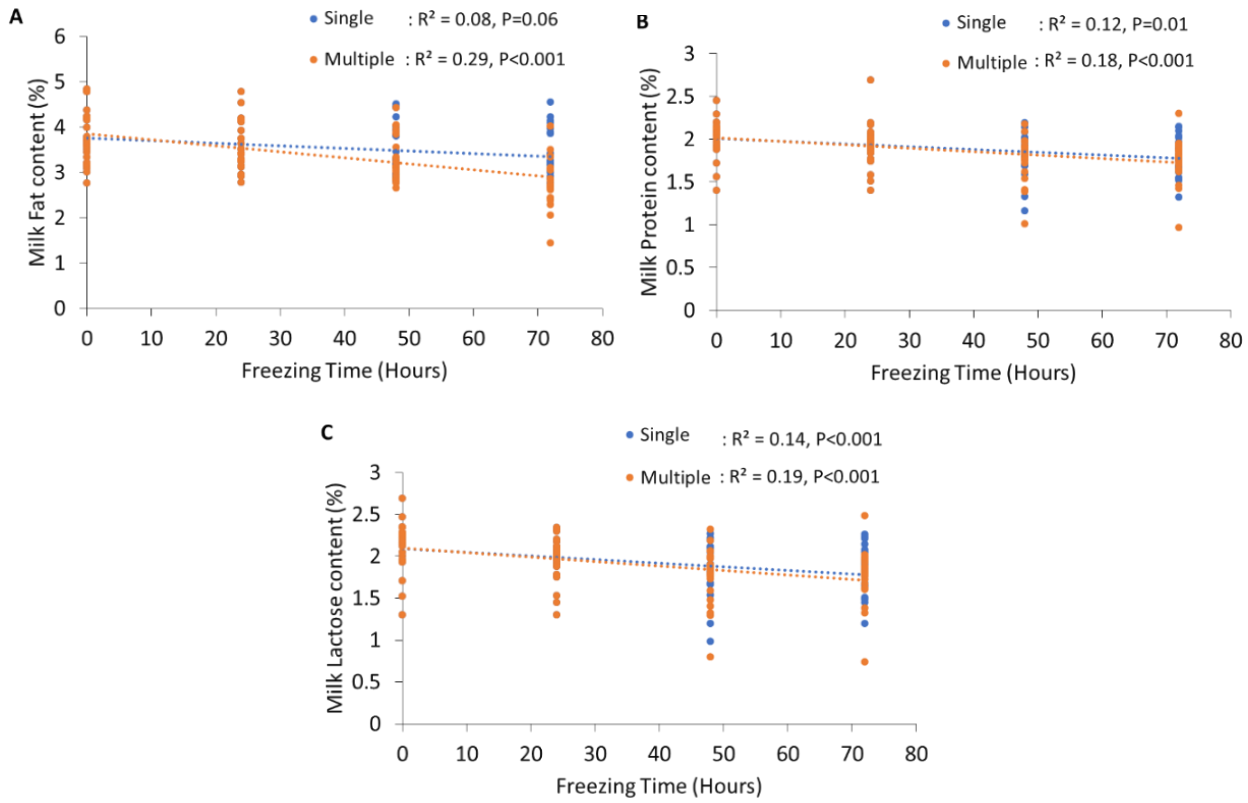

Figure 2. Correlation between duration of freezing (hours) and the changes in milk parameter content. Graph A is for Milk Fat, B is for Milk Protein, and C is for Milk Lactose.

Table 2. Interaction effect of freezing time and freezing types on milk composition of Friesian crossbred dairy cows.

Parameter	Time (hours)				P-Value		
	0	24	48	72	T	F	TXF
Fat (%)	3.81^{c}	3.59^{bc}	3.34^{ab}	3.14^{a}	<0. 001	0.03	0.02
Protein (%)	2.03^{b}	1.92^{b}	1.80^{a}	1.79^{a}	<0. 001	0.40	0.88
Lactose (%)	2.13^{c}	1.96^{b}	1.81^{ab}	1.80^{a}	<0. 001	0.40	0.86

T- freezing time, F- freezing type, TXF- Time and freezing type interactions, ^{abc}Mean with different superscripts within a row are significantly different (P<0.05).

4.5 and 7%, and 3.3 and 1.3% respectively (Haug et al., 2007; Martin et al., 2016). While the species influences the milk composition (Roessler et al., 2019), other factors like storage time and type (freezing or refrigeration) may affect the nutrient content.

Despite freezing fresh milk being a possible way of storing milk for a required duration, such an approach may have a deteriorating impact on product stability in terms of quality. Abranches et al. (2014), assessed the freezing and thawing effects on fat, protein, and lactose levels of natural human milk administered by gavage and continuous infusion. In the aforementioned study, there was a decrease in levels of milk parameters with increased freezing time. Similar results were observed in studies done by Weese et al. (1969) on effect of freezing and length of storage on dairy milk properties. The above studies demonstrated that refrigeration, freezing, and thawing lower macronutrient concentrations in human milk.

Previous studies have reported chemical and physical alteration of milk components during freezing and thawing (Abranches et al., 2014; García-Lara et al., 2012; Weese et al., 1969). In this study, milk fat decreased significantly with an increase in freezing time for single and multiple freezing experiments. However, this decline with freezing duration was more pronounced in the

multiple freezing experiments. These results were in congruence with other previous studies that reported depressed milk fat after cattle milk was stored at -26°C (Weese et al., 1969), goat milk frozen for 80 days at between −16 to −20°C (Yu et al., 2021), sheep milk frozen at -15 or -25°C (Zhang et al., 2006), and human milk samples frozen at -20°C (Abranches et al., 2014). Similar results were also reported in cattle milk where milk was refrigerated at 4°C (Rico et al., 2014; Zajác et al., 2015). Two hypotheses have been previously put forward to explain this result. First, when milk is stored at -20°C, the lipase activity goes on, albeit at a lower rate (Goff and Sahagian, 1996). Second, repeated thawing and freezing alters the fat globule by disrupting the globule membrane, increasing the substrate's accessibility to the depressed enzyme activity (Vieira et al., 2011). In these two theories, triglycerides in fat globules are broken down, lowering their content and increasing the content of diglycerides, monoglycerides, and free fatty acids. Since the monoglycerides, diglycerides, and free fatty acids are fats, it is expected that they could be measurable. Consequently, no significant differences were expected in the quantification of fat. It is theorized that the freezing and thawing circles could have facilitated further degradation of these molecules, therefore making them unmeasurable. However, this could not be confirmed as the equipment used could not determine the concentration of individual fat molecules in milk.

Similarly, milk protein and lactose had an apparent consistent decrease after freezing and thawing. This is in agreement with a study by Weese et al. (1969), who reported decreased fat, protein, and lactose in cattle milk. The decrease in milk constituents was attributed to coagulation and degradation when frozen milk is thawed. Specifically, the protein decrease was in agreement with a previous study by Păduraru et al. (2019) on the influence of refrigeration or freezing on human milk macronutrients. Another study by Vieira et al. (2011) reported up to 13.6% decrease in protein content when human milk was frozen and thawed. Several reasons have been put forward to explain the protein reduction. First, studies have suggested that milk protein flocculate and precipitate upon thawing after freezing (Babcock et al., 1949). In this study, flocculation was avoided by completely homogenizing the milk samples after thawing before taking the measurements. Secondly, during cold storage, casein micelles tend to lose their stability (Goff and Sahagian, 1996) because of physical aggregation after rejection from growing ice crystals and weakening of hydrophobic interactions between casein molecules within the micellar structure (Archer et al., 2017). Other studies have shown that, due to its crystallization ability during frozen storage, lactose is also an influential factor for casein destabilization. Goff and Sahagian (1996) reported that casein micelle destabilization could occur with as low as 40% lactose crystallization.

Conclusion

We conclude that despite freezing being a widely used storage technique, there is a significant decrease in fat, protein, and lactose content. This decrease in constituents was more pronounced when samples were frozen, thawed, and refrozen (multiple freezing) than when samples were thawed only once (single freezing).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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