Effects of Refrigeration, Freezing and Blast Freezing on Quality of Raw Cow's Milk

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Abstract

In the Philippines, milk production is often inconsistent due to seasonality leading to either shortage or surplus of milk. Before processing, dairy farmers and cooperatives subject their collected raw milk to low temperatures to delay the deterioration of milk quality. This process is performed to prevent profit loss, reduce food waste and generate a sustainable supply chain. The study determined the effects of refrigeration, freezing and blast freezing on preserving the quality of raw cow's milk before processing. Chemical, foaming, microbial and sensory characteristics were determined to compare the effect of each treatment. Milk samples were refrigerated at 4 °C for 12 h (RM), frozen at -18 °C for 16 h (FM), and blast frozen at -40 °C for 10 h (BFM). Before conducting the analyses, FM and BFM were thawed at 4 °C for 22 and 16 h, respectively. Fat (4.07%) and protein (2.97%) significantly decreased in FM while moisture, total solids, titratable acidity and pH did not significantly differ among treatments. The foam value and foam volume were significantly higher in RM (88.00% and 86.33 mL) and BFM (91.58% and 89.75 mL), respectively. BFM had the lowest counts of aerobic microorganisms (5.16 $\log_{10}$ CFU/mL), coliforms (1.38 $\log_{10}$ CFU/mL), and Escherichia coli (< 1.00 $\log_{10}$ CFU/mL). Milk samples were pasteurized at 63 °C for 30 min before the conduct of the sensory analysis. Sensory characteristics did not significantly differ among treatments. From the results, blast freezing can be generally considered the most viable solution for storing raw cow's milk before processing.

Keywords*:* cow's milk, blast freezing, freezing, milk quality, refrigeration

1. Introduction

Varying milk production is a usual problem encountered by dairy farmers and cooperatives in the Philippines. This can be caused by seasonality issues such as climate, pasture scarcity and reproductive performance (Pazzola *et al*.,

2013). During low production, raw milk is temporarily stored under low temperatures until a certain volume is met for collection and processing. The same procedure is also done to excess milk that cannot be fully absorbed by the market (Wendorff, 2001). These processes are practiced to manage milk production fluctuations, lessen fixed farm expenses and increase market availability (Tribst *et al*., 2019, 2020).

The backyard and small-scale dairy farmers typically use refrigeration as a method to preserve raw milk. After milking, immediate refrigeration of raw milk at 4 °C is carried out to ensure the production of safe and high-quality dairy products. At this temperature, the activity level of microorganisms and enzymes is low (Boubendir *et al*., 2016). However, temperature fluctuation and prolonged storage promote the proliferation of spoilage and pathogenic microorganisms. The maximum period in which refrigerated milk can be stored is dependent on the rapid temperature reduction after milking, initial microbial counts and refrigeration system (Fonesca *et al*., 2013; Yamazi *et al*., 2013).

In dairy processing, freezing is not limited to the manufacture of frozen dairy products but is also used in increasing the shelf life of raw milk. Despite the increasing popularity of new preservation techniques such as ultrasound, high-pressure, infrared irradiation and pulsed electric field, freezing is still generally the preferred process for shelf-life extension of food products. It may be attributed to its inexpensiveness, simplicity and conventionality (Tavman and Yilmaz, 2018). Freezing is considered a viable solution for increasing the interest in more sustainable supply chains globally (Baldwin, 2009). It decreases waste from spoilage and transportation delays which lowers the carbon footprint impact (Martindale, 2014). Moreover, frozen food products address seasonality challenges and have greater storability (Anaya *et al*., 2019). As the temperature lowers below the freezing point of milk, water in milk starts to nucleate and forms into large ice crystals as latent heat of crystallization is removed. Microbial damage or death may occur during freezing and frozen storage. These may be linked to the formation of large ice crystals that can puncture the cell walls of both bacteria and yeasts. The development of a supersaturated solution coupled with low water activity also hinders the growth of microorganisms (Alinovi *et al*., 2020). Freezing can mitigate inter-island milk supply by allowing the transport of frozen raw milk before further processing (Picon *et al*., 2013; Vélez *et al*., 2015).

Blast freezing is a rapid type of freezing wherein cold air is pushed at a high velocity across a food product (Campos *et al*., 2011; Greenquist, 2012). It produces small ice crystals that lessen physicochemical changes in the milk. The only limitation of using a blast freezer is its affordability. However, it has a higher preservation efficiency and effectivity in terms of time, space, energy and quality. When properly executed, blast freezing can potentially preserve the initial properties and nutrients of raw milk (Schafer, 2014). Subjecting raw milk to refrigeration and freezing are the common approaches used in prolonging its shelf life and preserving its quality. Hence, the objective of the study was to compare the chemical, foaming, microbiological and sensory qualities of raw milk subjected to refrigeration, freezing, and blast freezing.

2. Methodology

2.1 Experimental Design

Fresh raw cow's milk (2 L) was each subjected to refrigeration, freezing and blast freezing. For refrigerated milk (RM), the samples were immediately collected after subjecting the raw cow's milk to refrigeration (4 °C for 12 h). For frozen milk (FM) and blast frozen milk (BFM), the samples were immediately collected after every freezing-thawing process of raw cow's milk (FM: frozen at -18 °C for 16 h then thawed at 4 °C for 22 h; BFM: blast frozen at -40 °C for 10 h then thawed at 4 °C for 16 h). The temperature-time combinations were determined in a preliminary experiment. The study had five replications. Each replication had sample duplicates in every analysis.

2.2 Milk Collection and Preparation

Fresh raw cow's milk was provided by a dairy enterprise in Batangas, Philippines every replication during the experiment. The period of lactation, storage temperature, and storage duration were mid-lactation, 4 °C and < 4 h, respectively. Refrigeration and freezing of raw cow's milk were done using an upright refrigerator and freezer, respectively (Sharp SJ-T43R, Sharp Corporation, Philippines). Blast freezing of raw cow's milk was done using a blast freezer (CT Concepts AK-15D, CT Concepts, Philippines). Thawing of frozen and blast frozen raw cow's milk was carried out using an upright refrigerator (Sharp SJ-T43R, Sharp Corporation, Philippines).

2.3 Chemical Analysis

The treatment samples were tested for fat, protein, solids-not-fat and density using a calibrated milk analyzer (Ekomilk Ultra Milk Analyzer Milkana 98-2A, Bulteh 2000 Ltd., Bulgaria). Moisture and total solids content were determined using the oven method while titratable acidity (% lactic acid) was determined using the titration method (Association of Analytical Chemists [AOAC], 2006). The samples were tested for pH using a pH meter (Eutech pH 700, Eutech Instruments Pte. Ltd., Singapore).

2.4 Foaming Analysis

Each treatment sample (100 mL) was heated up to 75 °C in a double boiler with frequent stirring to distribute even heating temperature and prevent the formation of a protein film top layer. Each sample was poured into a milk frother (Milk Frother MLMF20, Modern Living and Milk Frother, Australia). The frothed milk was transferred into a 200-mL graduated cylinder to measure foam production. The method of Levy (2003) was followed to determine foam value (Equation 1), foam volume (Equation 2) and foam dissipation (Equation 3).

$$Foam\ value\ (FV) = \frac{100\ (TV - LV)}{LV} \tag{1}$$

$$Foam\ volume\ (FVol) = FH_5 - FI_5 \tag{2}$$

$$Foam\ dissipation\ (\%D) = \frac{IF - FH_5}{IF - FMI} \times 100 \tag{3}$$

where *TV* is the total volume of milk and foam; *LV* is the milk volume; FH_5 is the foam height after 5 min dissipation; FI_5 is the milk height after 5 min dissipation; *IF* is the foam height right after foaming; and *FMI* is the milk and foam interface.

2.5 Microbial Analysis

Serial dilutions from each treatment sample were prepared in sterile phosphate buffer saline diluents (pH 7.2) (HiMedia®, Mumbai, India) for aerobic microorganisms, coliform and *Escherichia coli* counts. All microbial counts were determined using a specific medium sheet (Sanita-kun® Aerobic Count and *E. coli*/Coliform Count Medium Sheet, JNC Corporation, Japan). The samples (1 mL) were each inoculated on the self-diffusible non-woven fabric

portion of the medium sheet. The medium sheets for aerobic microorganisms count and *E. coli*/coliform count were incubated at 35 °C for 48 h and 35 °C for 24 h, respectively. All aerobic microorganisms were detected as red-colored colonies while coliform and *E. coli* were noticed as blue/green and indigo/purple-colored colonies simultaneously.

A confirmation test for coliforms was performed by transferring at least 10 representative colonies to a tube of brilliant green lactose bile broth (BGLBB) (HiMedia®, Mumbai, India). The BGLBB tubes were incubated at 35 °C for 24 and 48 h to examine gas production. To confirm that the colonies were *E. coli*, at least 10 representative colonies were picked and transferred to a tube of *E. coli* broth (ECB) (HiMedia®, Mumbai, India). The ECB tubes were incubated at 44.5 °C for 24 and 48 h to examine gas production (Feng *et al*., 2018).

2.6 Sensory Analysis

Before the conduct of the sensory analysis, each treatment sample was vat (batch) pasteurized at 63 °C for 30 min (Chandan, 2016). Coded samples and drinking water were served to 10 trained panelists from the Dairy Training and Research Institute (DTRI), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna, Philippines. The panelists evaluated the samples for color, flavor, aroma, mouthfeel, creaminess, off-flavor and general acceptability using a linear scale of 0 to 100 (Mabesa, 1986). The bases for scoring were as follows: color – 0 (extremely undesirable) to 100 (extremely desirable); flavor – 0 (extremely undesirable) to 100 (extremely desirable); aroma – 0 (extremely undesirable) to 100 (extremely desirable); mouthfeel – 0 (extremely undesirable) to 100 (extremely desirable); creaminess – 0 (extremely undesirable) to 100 (extremely desirable); off-flavor – 0 (not perceptible) to 100 (highly perceptible); and general acceptability – 0 (extremely unacceptable) to 100 (extremely acceptable). Five sensory evaluation sessions were performed during the experiment treating each session as a replicate.

2.7 Statistical Analysis

Chemical, foaming, and microbial data of all samples were analyzed using analysis of variance (ANOVA) in a completely randomized design (CRD) while sensory analysis data were analyzed using the Kruskal-Wallis test. For each treatment, means were compared using pairwise mean comparison in

least significant difference (LSD). All statistical analyses were carried out using the SAS® University Edition software version SAS Studio 3.8 and SAS 9.4M6 (SAS Institute Inc., United States).

3. Results and Discussion

3.1 Chemical Compositions

The chemical compositions of RM, FM and BFM are presented in Table 1. Fat, solids-not-fat and density did not significantly differ between RM and BFM compared with FM. Regular freezing has a longer phase transition time which forms larger ice crystals that can cause fat globule destabilization. Expanding ice crystals disrupt fat emulsion resulting in the coalescence of fat (Tavman and Yilmaz, 2018). Fat globules are enclosed by milk fat globule membranes (MFGMs) which serve as protection against external damage. During freezing and frozen storage, MFGMs are ruptured through dehydration and the puncture of large ice crystals. This leads to creaming separation after thawing particularly the destabilization of emulsion and the partial coalescence of fat globules (Zhang *et al*., 2006; Tribst *et al*., 2019, 2020). Slow freezing also creates a supersaturated unfrozen solution. This increases the occurrence of chemical and enzymatic reactions due to the closer proximity of molecules. As result, fat oxidation, lipolysis and agglutinin activity are heightened. However, an increase in the concentration of enzyme inhibitors will also follow (Ashie *et al*., 1996).

Schafer (2014) and Fox *et al*. (2015) reported that rapid freezing of milk into thin blocks such as in blast freezing can lessen fat globule damages and ruptures. Blast freezing forms smaller ice crystals that are ineffective in perforating fat globules. Due to a shorter phase transition time and temperature, it also reduces the migration of unfrozen molecules leading to a solution with lower chemical and enzymatic activities. Milk fat is related to solids-not-fat (SNF) and density given in the empirical formula: SNF = 0.22 % fat + 0.25 density + 0.72. It proves that a decrease in fat or an increase in SNF will increase the density of the milk (Kailasapathy, 2016). Skimming or homogenization of fat before freezing can be recommended to reduce fat globule destabilization. However, the processability of thawed homogenized-frozen milk into value-added dairy products such as cheeses can be affected (Tribst *et al*., 2020).

Table 1. Chemical compositions of RM, FM and BFM

Components (%[1])	Treatments[2]			P-value
	RM	FM	BFM	
Fat	4.12±0.40[a]	4.07±0.40[b]	4.12±0.39[a]	0.0020
Protein	3.03±0.07[a]	2.97±0.08[b]	2.97±0.04[b]	0.0005
Solids-not-fat	8.55±0.33[b]	8.60±0.32[a]	8.56±0.34[b]	0.0026
Density (kg/m^3)	1.03±0.001[b]	1.03±0.001[a]	1.03±0.001[b]	0.0012
Moisture	87.34±0.32	87.33±0.33	87.33±0.32	0.5573
Total solids	12.66±0.32	12.67±0.33	12.67±0.32	0.5573
Titratable acidity (% lactic acid)	0.160±0.016	0.154±0.005	0.154±0.012	0.2705
pH	6.59±0.01	6.58±0.02	6.57±0.05	0.2440

[1] Except density and pH values; [2]([a, b]) means within rows having different superscripts are significantly different ($p < 0.05$).

Protein was significantly lower in FM and BFM compared with RM. Among the milk components, milk proteins specifically casein micelles are largely affected by freezing. As freezable water forms into ice crystals, the unfrozen solution increases in concentration and interaction leading to changes in the colloidal properties of milk proteins. Casein micelles particularly undergo micro-phase separation or cryoprecipitation (Fox *et al*., 2015). Interactions between proteins as well as protein aggregation occur due to the exposure of the hydrophobic portions of proteins (Xiong, 1997). These occurrences affect both the structural integrity and functional properties of proteins (Gaber *et al*., 2020). Protein aggregation can be attributed to an alteration in buffering capacity and ionic balance, precipitation of soluble minerals and formation of calcium-phosphate-casein complexes (Wendorff, 2001; Kljajevic *et al*., 2016; Tribst *et al*., 2019, 2020). The rate of freezing extensively affects the formation of freeze-induced protein aggregates. Slow freezing greatly favors aggregation than rapid freezing. The stability of regularly frozen milk can still be improved by a slow rate of thawing to allow re-solubilization of colloidal minerals (Gaber *et al*., 2020). The flocculation of casein is reversible with adequate agitation during thawing but can be permanent with long-term frozen storage. Cryoprecipitation allows casein micelles to release exogenous and indigenous proteases that attack the physical stability of proteins (Fox *et al*., 2015). Proteolysis will remain to transpire until the supersaturated unfrozen solution reached a glass state (Zhao and Takhar, 2017; Alinovi *et al*., 2020).

Moisture, total solids, titratable acidity and pH were not significantly different among treatments. It was reported that there was no significant difference between fresh or refrigerated milk and frozen milk samples in terms of pH,

titratable acidity, peroxide value and apparent viscosity (Tavman and Yilmaz, 2018). All values obtained were within the range of milk standards (Kailasapathy, 2016). Precipitation of soluble minerals may cause a minimal change in pH value. However, the results showed no significant changes in pH values. Psychrotrophic bacteria which can grow at low temperatures are not major acid-producer microorganisms (Martins *et al*., 2006).

Lactose content was not determined in this study. However, lactose crystallization happens during freezing which promotes instability through the conversion of β-lactose into a less soluble α-lactose monohydrate form. With the lower solubility of lactose, salting out occurs wherein lactose crystals are removed from the solution. This will lead to an increase in ice nucleation, ice crystal enlargement, and protein destabilization. Increasing the concentration of other milk components such as soluble carbohydrates can act as cryoprotectants that delay lactose crystallization. In addition, the production of highly soluble glucose and galactose from lactose hydrolysis has been reported to improve stability as they do not tend to crystallize during freezing. Rapid or blast freezing in combination with cold storage below -20 °C can slow lactose crystallization. On the other hand, slow or regular freezing can decrease the solubility index and increase the molecular mobility of the unfrozen phase. This will result in lower stability of milk during frozen storage (Alinovi *et al*., 2020).

3.2 Foaming Properties

The foaming properties of RM, FM and BFM are presented in Table 2. Stable milk foams are generated by balancing milk proteins and milk fat. Proteins, particularly β-casein, are surface-active agents with hydrophilic and hydrophobic regions that interact with the air and lipid phases of foam reducing surface tension (Fox *et al*., 2015). Aside from milk foam, milk proteins are important in ice cream and whipped cream manufacture. Agitation or whipping introduces air which leads to an unstable interface between air and water. Proteins with their surface-active components attach to the surface of air bubbles and stabilize them. The inclusion of some milk fat greatly contributes to flavor and mouthfeel. The heating of milk up to 75 °C is necessary to produce a stable foam. At > 40 °C, fat is liquified which then helps in overcoming the destabilizing effect of natural fat globules on foaming (Huppertz, 2014).

Table 2. Foaming properties of RM, FM and BFM

Properties	Treatments[1]			P-value
	RM	FM	BFM	
Foam value (%)	88.00±4.47[a]	80.62±7.01[b]	91.58±7.31[a]	0.0008
Foam volume (mL)	86.33±4.58[a]	78.12±7.52[b]	89.75±7.00[a]	0.0004
Foam dissipation (%)	6.49±1.70	7.77±3.07	6.79±2.83	0.1465

[1]([a, b]) Means within rows having different superscripts are significantly different ($p < 0.05$).

Foam value (FV) and foam volume (FVol) were significantly lower in FM than in RM and BFM. Regular freezing considerably promotes protein aggregation compared with blast freezing (Gaber *et al*., 2020). The structural integrity of milk proteins will be drastically affected leading to an unstable and minimal production of foam. On the other hand, Morr and Richter (1999) stated that refrigeration of milk caused physicochemical changes in casein micelles increasing viscosity and foaming tendency. Foam dissipation (%D) did not significantly differ among treatments which concurred with the observations of Gamboa and Barraquio (2012). They reported that the %D of different fat levels and age of milk were not significantly different.

3.3 Microbial Counts

The microbial counts of RM, FM and BFM are presented in Table 3. Microbial analysis is necessary to objectively assess milk quality. Food safety indicators of raw milk including aerobic microorganisms count, coliform count and the presence of pathogenic microorganisms such as *E. coli* should be determined (Hubáčková and Ryšánek, 2007). The aerobic microorganisms count of BFM was significantly lower than RM and FM. The results confirmed that freezing has microbiostatic and microbicidal effects resulting in cell damage and death, respectively (Food and Agriculture Organization [FAO], 2004). During freezing, crystallization gradually starts in the outer cells then water slowly migrates out due to osmotic pressure (Ray and Bhunia, 2014). The formation of intracellular and extracellular ice crystals causes cell dehydration, cell shrinkage and cell membrane damage leading to the increased mortality of microorganisms (El-Kest and Marth, 1992; Smith *et al*., 2011). These phenomena can be irreversible after thawing due to the damage to the cell structure. In a damaged cell membrane, internal leakage of cell materials such as RNA, DNA, potassium ions and low molecular solutes can lead to cell death. On the other hand, osmotic dehydration results in low water activity, limited cell mobility, cell deformation and nonlamellar lipid phase formation (Kennedy, 2000; Rahman, 2007; Sun, 2012).

Coliform and *E. coli* counts did not significantly differ among treatments. Subjecting milk to an adverse condition like freezing is more destructive to rod-shaped Gram-negative bacteria such as coliform and *E. coli* than spherical-shaped Gram-positive bacteria (Archer, 2004; Ray and Bhunia, 2014). These findings were consistent with those of Hubáčková and Ryšánek (2007) and Smith *et al*. (2011) who reported a decrease in coliform and *E. coli* counts after freezing. It should be noted that spore-forming bacteria such as *Clostridium* spp. and *Bacillus* spp. remain stable under freezing conditions and frozen storage. In addition, bacteria in the stationary phase are more stable than bacteria in the log phase.

Table 3. Microbial counts of RM, FM and BFM

Counts (Log_{10} CFU/ml)	Treatments[1]			P-value
	RM	FM	BFM	
Aerobic microorganisms	7.55^{a}	5.96^{b}	5.16^{c}	0.0428
Coliform	1.86	1.40	1.38	0.2701
E. coli	< 1.00	< 1.00	< 1.00	0.2637

[1]($^{a, b, c}$) Means within rows having different superscripts are significantly different ($p < 0.05$).

The thawing temperature is equivalently important in ensuring safe and high-quality milk. High and fluctuating thawing temperatures can promote the reversible recovery of injured microbial cells (Alinovi *et al*., 2020). Psychrotolerant and psychrotrophic microorganisms can also grow which can be detrimental. These microorganisms produce heat-resistant lipolytic and proteolytic enzymes that cause defects throughout storage (Pinto *et al*., 2006).

3.4 Sensory Characteristics

The sensory characteristics of pasteurized RM, FM and BFM are presented in Table 4. No significant differences among treatments were observed from the sensory parameters used. The sensory quality of milk is greatly affected by milk handling before processing. It can also be affected by several factors including feed quality, microbial contamination, chemical reaction, light exposure and thermal processing (Alvarez, 2016). Some studies reported that fat oxidation, lipolysis and proteolysis during freezing and frozen storage promoted off-flavor (Katsiari *et al*., 2002; Alinovi *et al*., 2020). A chalky mouthfeel can also be observed because of the precipitation of proteins and soluble minerals (Gaber *et al*., 2020).

Table 4. Sensory characteristics of pasteurized RM, FM and BFM

Sensory characteristics*	Treatments[1]			H value[2]	P-value
	RM	FM	BFM		
Color	80.44±3.11	80.90±2.50	78.20±2.11	4.02	0.1340
Flavor	77.12±2.37	80.94±2.50	75.88±1.36	4.46	0.1040
Aroma	77.84±1.74	80.36±1.44	79.10±3.20	4.96	0.0840
Mouthfeel	78.92±2.71	80.78±1.72	82.06±4.19	1.82	0.4025
Creaminess	76.26±3.92	79.18±2.70	79.42±2.88	2.54	0.2808
Off-flavor	4.25±1.94	6.87±1.14	6.26±3.78	2.66	0.2645
General acceptability	77.48±2.43	79.04±3.54	77.42±2.70	1.52	0.4677

[1] Means within rows having different superscripts are significantly different ($p < 0.05$); [2]means are significant if H values are greater than x^2 tab (x^2 tab = 5.9915).

4. Conclusion and Recommendation

Fat, solids-not-fat and density did not significantly differ between RM and BFM compared with FM. Protein was significantly lower in FM and BFM than RM. Moisture, total solids, titratable acidity and pH did not significantly differ among treatments. Foam value and volume were significantly lower in FM than in RM and BFM while foam dissipation did not significantly differ among treatments. The aerobic microorganisms count of BFM was significantly lower compared with RM and FM whereas coliform and *E. coli* counts did not significantly differ among treatments. The values for sensory characteristics were not significantly different among pasteurized treatments. The results from the analyses can be attributed to the effect of slow and rapid freezing on milk. Blast freezing has a shorter phase transition time producing smaller ice crystals and lesser saturated unfrozen solution. Generally, the measured values of BFM did not significantly differ from RM. This may suggest that blast freezing of milk can be considered the most suitable alternative to extend shelf life and address seasonality issues. The authors would like to recommend quantifying the ratio of β-lactose and α-lactose to determine the degree of lactose crystallization in milk during freezing. The presence of psychrotrophic microorganisms primarily *Pseudomonas* sp. and *Staphylococcus aureus* can also be determined to fully assess the microbial quality of the milk treatments. In addition, a cost analysis can be performed to compare the benefits and costs of using refrigeration, freezing and blast freezing in storing raw cow's milk.

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