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Physicochemical compositions of fluid milk products made from refrigerated, frozen-thawed, and blast frozen-thawed cow's milk

Ma. Czarina R. Moreno[1,*] and Olivia C. Emata[1,2]

1 Institute of Animal Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines
2 Dairy Training and Research Institute, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Laguna, Philippines

Abstract

Subjecting raw milk to low temperatures such as refrigeration and freezing are common practices by dairy farmers and cooperatives in the Philippines to slow down the deterioration of milk quality and address the fluctuations in milk production. However, physicochemical changes in milk including the formation of intracellular and extracellular ice crystals and the development of a supersaturated solution and concentration gradient occur during freezing. These changes may affect milk performance in the development of high-value-added dairy products. Hence, this study compared the physicochemical compositions of fluid milk products made from refrigerated, frozen-thawed, and blast frozen-thawed cow's milk (RM, FM, and BFM, respectively). For non-fat kefir, FM and BFM had significantly lower moisture content, higher total solids content, and higher viscosity compared to RM. Total solids content, lactic acid production, and viscosity are good indicators to be considered in producing high-quality kefir. For half-and-half cream, moisture content, total solids content, and viscosity did not significantly differ between RM and BFM. For light whipping cream, no physicochemical component was significantly different among treatments. Overall, based on the results of this study, blast freezing can be considered a suitable long-term storage condition of milk prior to further processing without negatively affecting its physical composition as fluid milk products.

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*Corresponding Author:
Ma. Czarina R. Moreno – *mrmoreno1@up.edu.ph*

Introduction

Milk and milk products are complex and nutrient-dense foods that provide energy and significant amounts of proteins and micronutrients. In particular, they give a major contribution to meeting the body's needs for important vitamins and minerals such as vitamin B_2 (riboflavin), vitamin B_5 (pantothenic acid), vitamin B_{12} (cobalamin), calcium, magnesium, and selenium (Muehlhoff et al., 2013).

The yield of milk every month in the Philippines is often inconsistent due to seasonality issues (Department of Agriculture, 2022). This leads to the temporary storage of collected raw milk under refrigeration or freezing temperatures until a specific volume is met for processing. Refrigeration of raw milk at 4°C immediately after milking is a standard procedure to significantly delay the increased activity of undesirable microorganisms and enzymes in milk (Boubendir et al., 2016; Chandan, 2016). Freezing of raw milk can also be considered a milk handling practice to impair microbial growth and reduce enzymatic reactions. In addition, the development of a supersaturated solution and a concentration gradient lowers water activity which inhibits microbial growth and motility (Alinovi et al., 2020). However, in terms of physicochemical components in milk, freezing can cause adverse changes which impose difficulties in processing milk into high-value-added fluid milk products such as kefir and cream (Tribst et al., 2020). The formation of intracellular and extracellular ice crystals during freezing can damage microbial cell walls and membranes (Smith et al., 2011).

Product development of milk into different products is essential in fully utilizing milk, maximizing profit, and keeping up with consumer preferences. Kefir is a fermented milk product that is slightly alcoholic and carbonated due to the metabolic activity of lactic acid bacteria, acetic acid bacteria, and yeasts in kefir grains or kefir starter culture when inoculated in milk (Farnworth, 2005). The large-scale and commercial production of kefir commonly uses direct-vat-inoculation (DVI) kefir starter culture due to difficulty in maintaining the viability of kefir grains. Similar to yogurt, kefir contains viable bacterial cells that encase β-galactosidase enzymes. These enzymes are responsible for promoting lactose digestion especially for lactose-intolerant consumers (de Vrese et al., 1992). It is considered a functional food due to its antimicrobial, antiviral, antifungal, anti-inflammatory, anticarcinogenic, and antimutagenic properties (Arslan, 2015; Farnworth, 2005).

The cream is the fat component of milk skimmed from its top layer. In commercial production, it is generally obtained from the centrifugal or mechanical separation of milk components. There are many types of creams based on the level of fat content and their usage. Half-and-half cream is a mixture of an equal proportion of milk and cream. It ranges from 10.5% to 18% fat content and is usually incorporated in coffee beverages as a substitute for a cream with high-fat content. On the other hand, light whipping cream contains 30% to 36% fat content (Chandan & Kilara, 2016). It can be used in baking, cooking, and beverage production. An important factor in producing cream products is its ability to form air cells during whipping and retain its shape without leakage of free fat (Walstra et al., 2006). The objective of the study was to compare the physicochemical compositions of fluid milk products (non-fat kefir, half-and-half cream, light whipping cream) made from refrigerated, frozen-thawed, and blast frozen-thawed cow's milk. Through this study, processors can determine if slow and blast freezing are suitable in preserving and storing milk without significantly affecting the physicochemical composition of its processed milk products.

Materials and Methods

Experimental Design

Non-fat kefir, half-and-half cream, and light whipping cream were made from refrigerated milk (RM), frozen-thawed milk (FM), and blast frozen-thawed milk (BFM). For each of the three experimental treatments, six liters of fresh raw cow's milk was used according to the following specifications.

RM - refrigerated at 4°C for 12 h (RM),
FM - frozen at -18°C for 16 h then thawed at 4°C for 22 h (FM), and
BFM - blast frozen at -40°C for 10 h then thawed at 4°C for 16 h (BFM).

Refrigeration, freezing, and thawing were carried out using an upright refrigerator and freezer (Sharp SJ-T43R, Sharp Corporation, Philippines). On the other hand, blast freezing was done using a blast freezer (CT Concepts AK-15D, CT Concepts, Philippines). The study had five replications with each replication having sample duplicates in every analysis.

Milk Collection and Preparation

A dairy enterprise in Batangas, Philippines provided fresh raw cow's milk every replication during the experiment. The stage of lactation, storage temperature, and storage duration were mid-lactation, 4°C, and <4 h, respectively. Each treated milk was pre-heated at 40°C to liquefy milk fat allowing the separation of non-fat milk and cream. Each treated milk was then poured into a cream separator to separate non-fat milk and cream. The non-fat milk was processed into non-fat kefir while the cream was divided into two portions: one portion was processed into half-and-half cream and the other portion was processed into light whipping cream.

Processing of Non-fat Kefir

The method of Moreno and Emata (2020) was carried out with some modifications. Non-fat cow's milk collected from RM, FM, and BFM was each pasteurized at 63°C for 30 min and then cooled down to 30°C. After cooling, a direct-vat-inoculation (DVI) kefir starter culture in freeze-dried format (Choozit™ Kefir DT, Danisco Deutschland GmbH, Germany) was inoculated in pasteurized non-fat cow's milk and left to incubate in a partially closed sterile container. The inoculation rate, incubation temperature, and incubation period were 0.5% (w/v), 23°C, and 18 h, respectively. These values were obtained from the manufacturer's technical memorandum (Danisco A/S, 2019). The final kefir product attained the standard pH (≈4.6) and titratable acidity (≈0.60%) (Food and Agriculture Organization [FAO], 2003). After incubation, the final kefir products were stored at 4°C for subsequent physicochemical analysis.

Processing of Half-and-half Cream and Light Whipping Cream

The fat content of cream collected from RM, FM, and BFM was each standardized to 11% to 12% for half-and-half cream and 33% to 34% for light whipping cream by the addition of non-fat milk. After standardization, a stabilizer (0.7% w/v, Recodan, Danisco Deutschland GmbH, Germany) was incorporated into the cream products to stabilize air cells during whipping. The products were homogenized-pasteurized at 63°C for 30 min. The light whipping cream samples were aged in a refrigerator at 4°C for 24 h to allow the coalescence of soft fat and hard fat. The aging process is important in the whippability and stability of the cream. The final cream products were stored at 4°C for physicochemical analysis (Chandan & Kilara, 2016).

Physicochemical Analysis

Protein (for non-fat kefir samples), fat (for half-and-half cream and light whipping cream samples), moisture and total solids, and titratable acidity (% lactic acid) of the final products were determined using Kjeldahl, Gerber, oven, and titration methods, respectively (Association of Analytical Chemists [AOAC], 2006). The pH and viscosity of the final products were determined using a pH meter (Eutech pH 700, Eutech Instruments Pte. Ltd., Singapore) and a viscometer (Brookfield Viscometer DV-I Prime, Brookfield Engineering Laboratories, Inc., USA), respectively.

Statistical Analysis

The physicochemical data of all samples were analyzed using Analysis of Variance (ANOVA) in a Completely Randomized Design (CRD). The means of each treatment were compared using Pairwise Mean Comparison in Least Significant Difference (LSD). All statistical analyses were processed using the SAS® University Edition software version SAS Studio 3.8 and SAS 9.4M6 (SAS Institute Inc., USA).

Results and Discussion

Physicochemical Analysis

The physicochemical compositions of non-fat kefir treatments are presented in Table 1. FM and BFM had significantly lower moisture content, higher total solids content, and higher viscosity compared to RM. These results can be attributed to higher exopolysaccharide (EPS) production in FM and BFM. Choozit™ Kefir DT contains a blend of kefir grain microflora, kefir yeasts (10^6-10^7 CFU·g^{-1}), and lactic acid bacteria (10^9-10^{10} CFU·g^{-1}) mainly isolated from kefir grains (Danisco A/S, 2019). Lactic acid bacteria particularly *Lactobacillus kefir*, *Lactobacillus parakefir*, and *Lactobacillus kefiranofaciens* produce exopolysaccharides called kefiran (Cheirsilp et al., 2003a, 2003b).

Table 1. Physicochemical compositions of non-fat kefir treatments.

Components (%[1])	Treatment[2]			*p*-value
	RM	FM	BFM	
Protein	2.10 ± 0.12	2.14 ± 0.10	2.00 ± 0.35	0.3848
Moisture	88.33 ± 0.69[a]	86.88 ± 1.44[b]	87.09 ± 1.25[b]	0.0214
Total solids	11.67 ± 0.69[b]	13.12 ± 1.44[a]	12.87 ± 1.25[a]	0.0192
Titratable acidity (% Lactic Acid)	0.62 ± 0.02	0.64 ± 0.02	0.64 ± 0.03	0.9545
pH	4.60 ± 0.03	4.60 ± 0.03	4.61 ± 0.04	0.9631
Viscosity, cP	1337.30 ± 31.82[b]	1371.70 ± 19.15[a]	1373.90 ± 14.78[a]	0.0020

[1]Except for pH and viscosity values
[2(a,b)]Means within rows having different superscripts are significantly different (p < 0.05)
RM - refrigerated milk
FM - frozen-thawed milk
BFM - blast frozen-thawed milk

Kefiran is comprised of D-glucose and D-galactose with a proposed structure of a branched hexa- or heptasaccharide unit to which one or two sugar residues are randomly linked (Arslan, 2015; Farnworth, 2005). It has been reported to improve the viscoelastic and rheological properties of acid milk gels at low temperatures avoiding water loss during storage. This makes it suitable to be used as an additive in fermented milk products as emulsifiers, stabilizers, gelling agents, and viscosity improvers. Kefiran demonstrated antiallergenic, antibacterial, antifungal, anti-inflammatory, antimutagenic, antioxidant, antitumor, antiulcer, and immunomodulatory properties. Some biological activities of kefiran include the reduction of high blood pressure and modulation of the intestinal immune system through the balancing of immune cells in the intestinal mucosa (Kök-Taş et al., 2012; Prado et al., 2015; Rosa et al., 2017).

Titratable acidity and pH did not significantly differ among treatments. However, freezing, whether slow or rapid, has microbiostatic and microbicidal effects resulting in cell damage and death (FAO, 2004). With lower microbial counts in milk due to freezing and pasteurization, there is less competition between kefir starter culture microorganisms and naturally occurring microorganisms. This leads to a higher conversion rate of lactose to lactic acid which lowers the titratable acidity and increases the pH value of kefir.

Protein did not significantly differ among treatments. However, refrigeration, freezing, and blast freezing of milk can cause changes in protein, particularly in casein micelles. Casein micelles undergo partial degradation which triggers the release of proteolytic enzymes. These enzymes are responsible for the slow coagulation and incomplete curd formation of milk during cheese processing (Morr & Richter, 1999).

Physicochemical Composition of Half-and-half Cream and Light Whipping Cream

Half-and-half Cream

The physicochemical compositions of half-and-half cream treatments are presented in Table 2. The results show that moisture content, total solids content, and viscosity did not significantly differ between RM and BFM. In terms of fat, Fox et al. (2015) stated that blast freezing of milk had minimal destabilizing effect on the lipoprotein complexes of fat globules due to the formation of small ice crystals, whereas slow freezing of milk had detrimental effects on the physicochemical properties of fat globules and fat globule membrane proteins which include the absorption of whey proteins that promote creaming. Furthermore, slow freezing promotes lipase-catalyzed rancidity and develops oxidative off-flavors (Morr & Richter, 1999).

Table 2. Physicochemical compositions of half-and-half cream treatments.

Components (%[1])	Treatment[2]			*p*-value
	RM	FM	BFM	
Fat	11.38 ± 0.10	11.23 ± 0.12	11.11 ± 0.03	0.9906
Moisture	84.33 ± 0.42[a]	83.48 ± 0.58[b]	84.16 ± 0.91[a]	0.0207
Total solids	15.67 ± 0.42[b]	16.52 ± 0.58[a]	15.84 ± 0.91[b]	0.0207
Titratable acidity (% Lactic Acid)	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.9736
pH	6.61 ± 0.02	6.63 ± 0.03	6.60 ± 0.01	0.9573
Viscosity, cP	1314.80 ± 26.10[b]	1347.70 ± 24.25[a]	1328.80 ± 16.12[ab]	0.0113

[1]Except for pH and viscosity values
[2(a,b)]Means within rows having different superscripts are significantly different ($p < 0.05$)
RM - refrigerated milk
FM - frozen-thawed milk
BFM - blast frozen-thawed milk

During slow freezing, loss of moisture can happen due to the crystallization of free water (Schafer, 2014). An increase in titratable acidity or a decrease in pH can also occur due to a "salting out" action wherein calcium caseinates are released from their colloidal dispersion and into the high concentration of milk salts in the unfrozen portion of milk. Milk salts particularly calcium phosphates, disodium phosphates, and sodium carbonates embedded in casein micelles are also precipitated which leads to a decrease in pH (Fox et al., 2015). The viscosity of milk and milk products can be affected by the concentration and physical state of fats and proteins along with temperature changes. Physicochemical changes in fat globules and casein micelles during refrigeration and freezing can increase the viscosity and foaming tendency of cream. Cream is considered a non-Newtonian fluid at <40°C due to its ability of shear thinning and thickening (Kailasapathy, 2016). Increasing the fat content and/or reducing the temperature favors non-Newtonian behavior (Fox et al., 2015).

Light Whipping Cream

The physicochemical compositions of light whipping cream treatments are presented in Table 3. No physicochemical component was significantly different among treatments. It is possible that physical defects including oiling off, cream plugs, and age thickening may occur in high-fat cream products. This is due to the high levels of free fat wherein milk fat globule membranes are totally or partially removed resulting in fat leakages (Fox et al., 2015). Stability may be improved by the aging of cream at refrigeration temperature (4°C) for 24 h after processing. This induces the coalescence of soft and hard fat which is essential in the ability of the cream to retain its shape during whipping (Walstra et al., 2006).

Table 3. Physicochemical compositions of light whipping cream treatments.

Components (%[1])	Treatment[2] RM	FM	BFM	*p*-value
Fat	33.90 ± 0.61	33.70 ± 0.83	33.60 ± 0.72	0.6486
Moisture	57.92 ± 0.31	57.39 ± 0.80	58.05 ± 0.87	0.1076
Total solids	42.08 ± 0.31	42.61 ± 0.80	41.95 ± 0.87	0.1076
Titratable acidity (% Lactic Acid)	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.9652
pH	6.60 ± 0.03	6.60 ± 0.02	6.58 ± 0.02	0.1543
Viscosity, cP	2287.40 ± 18.39	2318.10 ± 27.52	2303.80 ± 36.68	0.0723

[1]Except for pH and viscosity values
[2(a,b)]Means within rows having different superscripts are significantly different ($p < 0.05$)
RM - refrigerated milk
FM - frozen-thawed milk
BFM - blast frozen-thawed milk

Conclusion

This study reported that the refrigeration, freezing, and blast freezing of milk prior to processing can affect the physicochemical compositions of its products (non-fat kefir, half-and-half cream, and light whipping cream). Non-fat kefir made from FM and BFM did not significantly differ in terms of moisture, total solids, and viscosity. It can be suggested that slow or blast freezing of milk before non-fat kefir production are both suitable means of storage without major comparable changes. Meanwhile, half-and-half and light whipping creams produced from RM and BFM were generally not significant from each other. It can be proposed that blast freezing is more appropriate for preserving and storing milk than slow freezing. Several studies reported that slow freezing has more detrimental effects on the structure of fat globules and fat globule membrane proteins than blast freezing. This was due to the larger formation of ice crystals during freezing. Overall, based on the results of this study, blast freezing can be considered a suitable long-term storage condition of milk prior to further processing without negatively affecting its physical composition as fluid milk products. Microbial counts, sensory characteristics, and other quality parameters of the treatments can also be analyzed in future studies. Direct freezing and blast freezing of fluid milk products can also be conducted to determine the full extent of these storage treatments on milk components.

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Disclosure Statement

No potential conflict of interest was declared by the authors.

References

Alinovi, M., Mucchetti, G., Wiking, L., & Corredig, M. (2020). Freezing as a solution to preserve the quality of dairy products: The case of milk, curds and cheese. *Critical Reviews in Food Science and Nutrition*, *61*(20), 3340-3360.

Arslan, S. (2015). A review: chemical, microbiological and nutritional characteristics of kefir. *CyTA–Journal of Food*, *13*(3), 340-345.

Association of Analytical Chemists (AOAC). (2006). *Official methods of analysis of AOAC International*. Rockville, MD: AOAC International.

Boubendir, A., Serrazanetti, D.I., Hamidechi, M.A., Vanini, L., & Guerzoni, M.E. (2016). Changes in bacterial populations in refrigerated raw milk collected from a semi-arid area of Algeria. *Annals of Microbiology*, *66*, 777-783.

Chandan, R.C. (2016). Dairy processing and quality assurance. In R.C. Chandan, A. Kilara, & N.P. Shah (Eds.). *Dairy processing and quality assurance* (p. 5). West Sussex, UK: John Wiley & Sons Ltd.

Chandan, R.C., & Kilara, A. (2016). Dairy-based ingredients. In R.C. Chandan, A. Kilara, & N.P. Shah (Eds.). *Dairy processing and quality assurance* (p. 202). West Sussex, UK: John Wiley & Sons Ltd.

Cheirsilp, B., Shimizu, H., & Shioya, S. (2003a). Enhanced kefiran production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*. *Journal of Biotechnology*, *100*(1), 43-53.

Cheirsilp, B., Shoji, H., Shimizu, H., & Shioya, S. (2003b). Interaction between *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae* in mixed culture for kefiran production. *Journal of Bioscience and Bioengineering*, *96*(3), 279-284.

Danisco. (2019). *Technical Memorandum TM 2082-1e Danisco kefir cultures*. Retrieved September 20, 2022, from https://cuisinedocbox.com/docview/83/88004818/#file=/storage/83/88004818/88004818.pdf

de Vrese, M., Keller, B., & Barth, C.A. (1992). Enhancement of intestinal hydrolysis of lactose by microbial β-galactosidase (EC 3.2.1.23) of kefir. *British Journal of Nutrition*, *67*(1), 67-75.

Department of Agriculture (DA). (2022). Philupdates. Retrieved September 20, 2022, from https://nda.da.gov.ph/dairy-atlas-2/

Farnworth, E.R. (2005). Kefir – a complex probiotic. *Food Science and Technology Bulleting Functional Foods*, 2(1), 1-17.

Food and Agriculture Organization (FAO). (2003*). Standard for fermented milk (CXS 243-2003)*. Retrieved September 20, 2022, from http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B243 2003%252FCXS_243e.pdf

Food and Agriculture Organization (FAO). (2004). *Code of hygienic practice for milk and milk products (CAC/RCP 57-2004)*. Retrieved September 20, 2022, from http://www.fao.org/fileadmin/user_upload/livestockgov/documents/CXP_057e.pdf

Fox, P.F., Uniacke-Lowe, T., McSweeney, P.L.H., & O'Mahony, J.A. (2015). *Dairy chemistry and biochemistry* (p. 115, 116, 122, 153, 222, 265.). Cham, Switzerland: Springer.

Kailasapathy, K. (2016). Chemical composition, physical, and functional properties of milk and milk ingredients. In R.C. Chandan, A. Kilara, & N.P. Shah (Eds.). *Dairy processing and quality assurance* (p. 95). West Sussex, UK: John Wiley & Sons Ltd.

Kök-Taş, T., Seydim, A.C., Ozer, B., & Guzel-Seydim, Z.B. (2012). Effects of different fermentation parameters on quality characteristics of kefir. *Journal of Dairy Science*, *96*(2), 780-790.

Moreno, M.C.R. & Emata, O.C. (2020). Chemical and microbiological properties of kefir produced by kefir grains in raw and pasteurized cow's milk. *Mindanao Journal of Science and Technology*, *18*(2), 277-292

Morr, C.V. & Richter, R.L. (1999). Chemistry of processing. In N.P. Wong, R. Jenness, M. Keeney, & E.H. Marth (Eds.), *Fundamentals of dairy chemistry* (pp. 740-741). New York: Springer.

Muehlhoff, E., Bennett, A., & McMahon, D. (2013). Introduction. In E. Muehlhoff, A. Bennett, & D. McMahon (Eds.), *Milk and dairy products in human nutrition* (p. 5). Rome, Italy: Food and Agriculture Organization of the United Nations.

Prado, M.R., Blandón, L.M., Vandenberghe, L.P.S., Rodrigues, C., Castro, G.R., Thomaz-Soccol, V., & Soccol, C.R. (2015). Milk kefir: composition, microbial cultures, biological activities, and related products. *Frontiers in Microbiology*, *6*, 1177.

Rosa, D.D., Dias, M.M.S., Grześkowiak, Ł.M., Reis, S.A., Conceição, L.L., & Peluzio, M.D.C.G. (2017). Milk kefir: nutritional, microbiological and health benefits. *Nutrition Research Reviews*, *30*(1), 82-96.

Schafer, W. (2014). *The science of freezing foods*. Retrieved September 20, 2022, from https://www.extension.umn.edu/food/food-safety/preserving/freezing/the-science-of-freezing-foods/

Smith, E.M., Monaghan, E.M., Huntley, S.J., & Green, L.E. (2011). Short communication: Preliminary investigation into the effect of freezing and a cryopreservant on the recovery of mastitis pathogens from ewe milk. *Journal of Dairy Science*, *94*(10), 4850-4855.

Tribst, A.A.L., Falcade, L.T.P., Carvalho, N.S., Cristianini, M., Leite Júnior, B.R.C., & de Oliveira, M.M. (2020). Using physical processes to improve physicochemical and structural characteristics of fresh and frozen/thawed sheep milk. *Innovative Food Science and Emerging Technologies*, *59*, 102247.

Walstra, P., Wouters, J.T.M., & Geurts, T.J. (2006). *Dairy science and technology* (p. 452). Boca Raton: FL: CRC Press.