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Comparison of Physicochemical and Sensory Properties of Freeze-concentrated Milk with Evaporated Milk during Storage

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ABSTRACT : This study was carried out to compare the changes of nutrients, sensory and chemical properties of freeze-concentrated and evaporated milks during storage. For pasteurization, the freeze-concentrated milk containing 27% of total solid was treated with 150 rpm ozone for 5 min, and 99% of microflora was eliminated. Also, the activities of protease and lipase decreased 93.31% and 96.15%, respectively, and phosphatase showed negative activity. Total bacteria count was maintained below 2.0×10^4 CFU/ml. During storage, TBA absorbance was lower in freeze-concentrated milk than that in the evaporated milk. The production of short-chain free fatty acids and free amino acids increased proportionally to the storage period in both samples. While the short-chain free fatty acid production was lower in the freeze-concentrated milk compared with that in the evaporated milk, the production of individual free amino acid was similar in both samples. In sensory evaluation, cooked flavor and color were much lower in the freeze-concentrated milk than that in the evaporated milk. Overall acceptability score was higher in the freeze-concentrated than the evaporated milk. Based on above results, ozone treatment for the freeze-concentrated milk pasteurization was positive at the elimination of microflora and enzyme inactivation. During storage, the freeze-concentrated sample minimized the change of color and TBA absorbance, the production of short-chain free fatty acid and vitamins than the evaporated milk. Therefore, the freeze-concentrated milk process in the present study resulted in the positive effect in minimizing nutrient loss and keeping quality of milk during storage. (**Key Words :** Freeze-concentrated Milk, Evaporated Milk, Milk Nutrients, Ozone Treatment)

INTRODUCTION

As in much of the food industry, and indeed society, economics largely determine the use of milk in the fresh fluid form or in some concentrated and preserved form. Concentrated milk products occupy less space, weigh less, and keep shelf-life longer than fresh milk. Hence, they save storage and packaging space, cost less to transport, and serve as a reserve in time of short supply. Concentrated sources of milk solids are required for formulating numerous foods which would be diluted by a less concentrated form of milk (Campbell and Marshall, 1975a).

In the dairy foods, concentrated milk has been widely used as intermediate materials or final products for consumers. To obtain a concentrate, the water content in milk must be reduced by 75%, and so there should be some operation to eliminate water (Speer and Mixa, 1998). In concentrations of liquid food, there are generally three methods, the evaporation; reverse osmosis; freeze concentration (Miyawaki et al., 2005). In these methods, the evaporation process is mainly used for the concentrated milk, but there are some problems, like discoloration, heat-coagulation and burnt flavor in the final products due to thermal processing. In addition, when milk is heated at temperature above 70°C, the whey protein is denatured and will interact with other milk proteins (Anema et al., 2004). Therefore, freeze concentration is being recently studied as a non-thermal processing technology in the dairy industry.

The application of freeze concentration to the dairy industry has been demonstrated in the past (Van Mil and Bouman, 1990); however, only limited commercial success has been seen. Although freeze-concentrated milk is aimed at replacing existing products as well as for future product developments in the dairy industry (Hartel and Espinel, 1993), there have been few researches about the freeze-concentrated milk with limited technology, the present study investigated changes of nutrient composition, and chemical and sensory properties of the freeze-concentrated

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milk by the newly developed process of multi-stage freeze concentration.

The quality of unsweetened condensed milk is altered during storage. The rate of change depends on the applied time-temperature regime. These alterations are more intensive at higher temperatures and after longer storage periods (Caric, 1994a). In 1990s, minimally processed, fresh-like products with reduced thermal inputs during sterilization and preservation have become commonplace in the food industry (Mertens and Knorr, 1992). Among nonthermal food preservation processes including electric or magnetic fields, microwave radiation, ionizing radiation, light pulses, high isobaric pressures, and chemical agents such as carbon dioxide (Graham, 1997), ozone treatment was chosen for sterilization of the contaminated microorganisms.

Ozone is polymerized oxygen with a molecular weight of 48. Since molecular ozone is highly effective for inactivating many microorganisms, such as bacteria and viruses (Driedger et al., 2001), ozone has been usually used in water and waste water disinfection and in the preservation of food products to provide microbiologically safe (Broadwater, 1973; Yang and Chen, 1979; Graham, 1997; Xu, 1999). Also, ozonation is recognized by the Food and Drug Administration as a disinfectant or sanitizer for foods (Graham, 1997).

However, since limited commercial skill and regulation in the world dealing with the use of ozone in foods is available, there has been no opportunity for commercial vendors to develop skills with food products. Therefore, the objectives of this study were to examine the changes of freeze-concentrated milk on physicochemical and sensory properties during storage comparing with evaporated milk.

MATERIALS AND METHODS

Freeze concentration process

The freeze concentration was progressed in the multi-stage freeze concentrator (Park et al., 2006). The initial structure of the concentrator could be explained as follows. Ice crystals were formed on the inner wall of the stainless vessel by circulating coolant from the cryostat (FP-80, Julabo, Seelbach, Germany). Teflon scrapper continually rotated with 50-60 rpm in the center of concentration vessel to remove ice crystals maintaining the minimum distance (15 mm) from the inner wall. Removed ice crystals gathered to the central part and recrystallized. Coolant temperature was varied with the regular procedure to accelerate the recrystallization of ice, and this operation was defined as a heat and cold shock in our study. Supercooling was observed to the sample temperature of -4°C in the beginning of freezing process. Initial freezing occurred at sample temperature of -4°C coupled with nucleation and crystallization, and then there was the increment of sample temperature to -0.3°C due to latent heat during crystallization process. After nucleation, coolant temperature increased to -2°C as heat shock operation and maintained constantly in the recrystallization process. Sample temperature indicated -0.3°C in the early stage and slowly decreased to -1.8°C at the end of recrystallization process. The temperature range was intended to let sample have its' temperature near the freezing point in the middle of recrystallization course. Temperature profiles of coolant and milk were collected with K-type thermocouple and data logger (MV-100, YOKOGAWA, Tokyo, Japan). After each recrystallization process, ice crystals and milk concentrates were separated by filtration process coupled with stainless steel net (200 mesh) and vacuum pump. In this experiment, the multi-stage process (one and two stage) was conducted to increase the concentration.

Ozone treatment

To pasteurize the freeze-concentrated milk, samples were treated by ozone generator (Korea Ozone Tech., AOP type, Seoul, Korea) which oxygen was produced from purified, extra dry oxygen. The ozone generator was connected to a 200 ml washing bottle, which contained 100 ml of the freeze-concentrated milk, and bubbled for 1, 3 and 5 min at 25°C. The ozone generator was operated by using UV light with 187 nm wavelength. Dry oxygen concentration was 150 ppm (0.72 mg/L), pressure was 0.5 kg/cm, and flow rate was 7 L/min. Other samples were not ozone-treated and all treatments were triplicated.

Microbial test

Microbial counts were determined from the colony counts on PCA agar. Freeze-concentrated milk was treated with 150 rpm of ozone for 1, 3 and 5 min, and stored for 18 days. One milliliter of milk sample stored for 0, 6, 12 and 18 day was diluted with 9 ml of sterile peptone and water diluent. Subsequent serial dilutions of each sample were plated in triplicate and incubated at 35°C for 48 h.

Measurement of enzyme activities

To examine how effective the ozone treatment on enzyme activity, the activities of protease, lipase and phosphatase were measured (Chae et al., 2002).

Evaporation process

The evaporated milk was prepared to have the same solute concentrate with the freeze concentrated milk through the vacuum evaporator (R-205, Büchi, Bern, Swiss). During evaporation process, the vapor temperature maintained 70°C to prevent the major changes in milk component under the vacuum state of 300 torr.

Chemical composition

Milk samples were analyzed for moisture, fat, protein and ash using the methods of Association of Official Analytical Chemists (AOAC). The content of lactose in milk was determined by Kwak and Jeon (1988). Sample (10 g) was poured into a 25 mL volumetric flask, and 2-propanol was filled and mixed thoroughly. It was stood at room temperature for 20 min and centrifuged at 275×g for 10 min, and the supernatant was filtered through Whatman no 540 and Sep-Pak C_{18} for HPLC determination. Lactose analysis was performed by using a Cosmosil packed column 10 NH_2 (4.6 mm I.D.×25 cm), and HPLC (Waters Corporation, MA, USA). Acetonitril:water (3:1) was used as a mobile phase and propelled at 2 ml/min. Detector was refractive index detector and injector volume was 20 μl. A standard curve was constructed by injecting glucose and galactose standards, which yielded a linear curve. All measurements were run in triplicate.

Thiobarbituric acid (TBA) test

Oxidation products were analyzed spectrophotometrically using the TBA test (Hehenauer et al., 1979). The TBA reagent was prepared immediately before use by mixing equal volumes of freshly prepared 0.025 M TBA, which was neutralized with NaOH and 2 M H_3PO_4/2 M citric acid. Reactions of the TBA test were started by pipetting 5.0 ml of milk samples into a glass centrifuge tube and mixed thoroughly with 2.5 ml of TBA reagent. The mixture was heated immediately in a boiling water bath for exactly 10 min and cooled on ice. Ten milliliters of cyclohexanone and 1 ml of 4 M ammonium sulfate were added and centrifuged at 2,490×g for 5 min at room temperature. The orange-red cyclohexanone supernatant was decanted and its absorbance at 532 nm measured spectrophotometrically in a 1-cm light path. All measurements were run in triplicate.

Short-chain free fatty acids (FFAs)

Milk samples (1 ml) were removed periodically, and extracted with diethylether and hexane for 2 h and eluted through a 10 mm I.D. glass column containing neutral alumina as described by Ikins et al. (1988). A Hewlett-Packard Model 5880A GC equipped with a flame ionization detector as used. The preparation of FFA was achieved using a 15 m×0.53 mm I.D. Nukol fused-silica capillary column (Supelco Inc., Bellefonte, PA, USA). The GC was operated with helium carrier gas at 2 ml/min, hydrogen gas 37 ml/min, and air at 300 ml/min. The column oven was programmed as an initial holding for 1 min at 110°C and first level holding to 180°C at 5°C/min for 10 min and holding for 20 min. Both temperatures for injector and detector were 250°C. All quantitative analyses were done by relating each peak area of individual FFA to the peak area of tridecanoic acid as an internal standard. Each FFA was identified by the retention time of standard.

Free amino acids

To determine free amino acid (FAA), 5 g of milk was mixed with 5 ml distilled water. Then 500 mg of sulfosalicyclic acid was added to the mixture, after which the mixture was stored at 4°C for 1 h and centrifuged at 1,300×g for 15 min. The supernatant was filtered through a 0.45 μm filter paper and pre-treated by the method described by Lindorth and Mopper (1979). Determination of FAA by using HPLC was done by the modified method of Hodgin et al. (1983). Flow rate was 2 ml/min and two mobile phases were used: solvent A was 0.05 M sodium acetate (pH 6.3), and solvent B, methanol:THF (90:10, v/v). The linear gradient of solvent B was programmed at 5 levels as follows: initial starting at 20%, then increasing of 40% for 6 min, to 42% for 9 min, to 50% for 3 min and finally to 70% for 12 min. FAA was analyzed on an ODS-μ-Bondapak C column (3.9 mm×30 mm), and a HPLC (Waters, Plymouth, MN, USA) equipped with a RI detector was used. All quantitative analyses were performed by relating peak areas of individual FAA to those of external standard amino acids (Wako, Osaka, Japan). All samples were analyzed in triplicate.

Vitamins

To determine water soluble vitamins, 0.5 ml of milk was placed in 50 ml volumetric flask and mixed with mobile phase and sonicated for 20 min. The mixed solution was centrifuged at 452×g for 20 min, filtered and injected into HPLC. Vitamins were analyzed on Shodex RSpak DE-413L column (4.6 mm×250 mm), and a HPLC (Waters, Plymouth, MN, USA) equipped with a RI detector was used. Flow rate was 0.5 ml/min and two mobile phases were used: solvent A was 0.005 M PIC B_6, and solvent B, the mixture of 0.4 ml triethylamine in 15 ml methanol:acetic acid (1:1, v/v). The linear gradient of solvent B was programmed at 5 levels as follows: initial starting at 20%, then increasing to 40% for 6 min, to 42% for 9 min, to 50% for 3 min and finally to 70% for 12 min. All quantitative analyses were performed by relating peak areas of individual vitamins to those of external standards (Wako, Osaka, Japan). All samples were analyzed in triplicate.

To determine fat soluble vitamins, 1.0 ml of milk was placed in screw-capped glass tube, added by 1 ml of 10% pyrogallic acid/ethanol mixture, mixed with 9 ml of KOH solution and sonicated for 30 min. After cooling to room temperature, fat-soluble vitamins were extracted with 30 ml ether and 30 ml distilled water. The process was repeated three times. The ether layers were transferred to a round-bottom flask and dried under vacuum. The extract was re-dissolved in 10 ml isopropanol, filtered and injected into HPLC.

Table 1. Effect of ozone treatment on microbial count and phosphatase activity in freeze-concentrated milk[1]

Treatment	Time of ozone treatment (min)			
	0	1	3	5
	CFU/ml			
Microbial counts	$2.5\times10^{5}\pm0.2^{a}$	$8.5\times10^{4}\pm0.2^{ab}$	$5.3\times10^{4}\pm0.2^{b}$	$4.0\times10^{3}\pm0.2^{c}$
Phosphatase acitivity	+	+	+	-

[1] Means by the same letter are not significantly different ($p<0.05$).
Ozone concentration was 150 ppm at 7 L/min air flow rate.

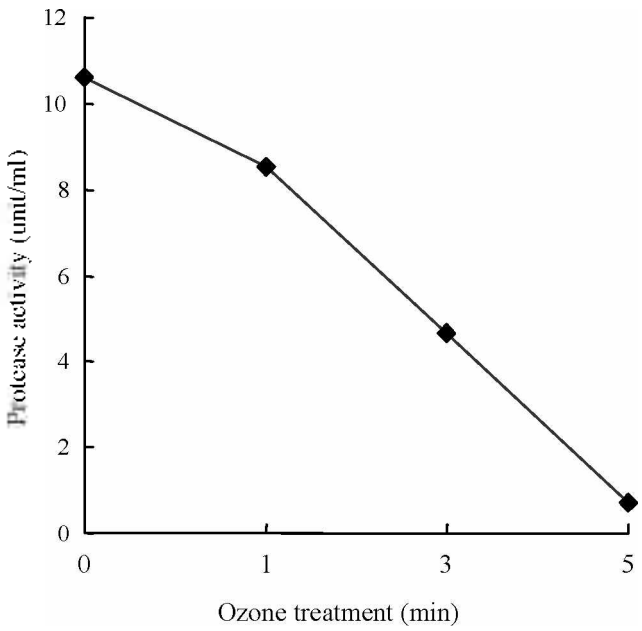

Figure 1. Effect of ozone treatment on protease activity in freeze-concentrated milk. Ozone concentration was 150 ppm at 7 L/min air flow rate.

Color

Color values were compared between the freeze concentrated and the evaporated milk with the colorimeter (CR210, Minolta, Tokyo, Japan) after calibrating its original value with standard plate (X = 97.83, Y = 81.58, Z = 91.51). Measured L, a, and b values were regarded as indicator of lightness, redness and yellowness, respectively.

Sensory analysis

For the sensory test, ten-trained panelists evaluated randomly coded milks. The intensities of cooked and oxidized flavor and fat, oxidized and off-taste were evaluated on a 7-point scale (1 = very slight, 2 = slight, 3 = slight-moderate, 4 = moderate, 5 = moderate-strong, 6 = strong, and 7 = very strong). Overall preference was scored on a 7-point scale (1 = like extremely, 4 = neither like nor dislike and 7 = dislike extremely). A randomized, balanced and complete block design was used (Cochran and Cox, 1957) in two replications for all samples.

Statistical analysis

Data from the determination of optimum conditions of samples, one-way ANOVA (SAS, 1985) was used. The significance of the results was analyzed by the least significant difference (LSD) test. Difference of $p<0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Pasteurization of freeze-concentrated milk by ozone treatment

For pasteurization of freeze-concentrated milk, we tried to apply ozone treatment, since heat sterilization could not be appropriate. As a preliminary study, 150 rpm of ozone was treated for 1, 3 and 5 min, and microbial counts and enzyme activities were examined.

Microbial counts : The presence of microorganisms observed after ozone treatment was shown in Table 1. When 150 ppm ozone was applied for 1 min, total count was reduced to 66.0% (8.5×10^{4} CFU/ml) of an initial count. With 3 and 5 min treatments, the microbial counts were reduced to 78.8 and 98.4%, respectively. Therefore, only ozone treatment for 5 min may result in an effective pasteurization of freeze-concentrated milk.

In previous study (Kwak et al., 2002), we examined the effects of ozone treatment in microencapsulated lactase on sterilization of microorganisms during storage of milk. This study indicated that 10 ppm ozone treatment reduced microbial counts by about 70% from the initial values. Broadwater et al. (1973) indicated that ozone, in relatively low concentration, was an effective bacteriocide against both vegetative cells and spores of three bacterial species. In practical application, ozone treatment probably could be applied at higher dosages (0.5-10 ppm) and for longer contact periods (2-10 min) because, as was shown in their research, organic matter present in the water will exert an ozone demand and prevent the full utilization of the applied dose as a disinfectant.

Enzyme activities : The protease activity by the ozone treatment was shown in Figure 1. The protease activity decreased slowly up to 1 min, and reduced sharply thereafter. The protease activities were 10.61 and 0.71 unit/ml at 0 and 5 min treatments, respectively. The similar trend of decrease was found in lipase activity (Figure 2). With 5 min of ozone treatment, the lipase activity was reduced to 92.9% from 0.70 unit/ml (0 min) to 0.05 unit/ml (5 min). The phosphatase activity was disappeared with 5

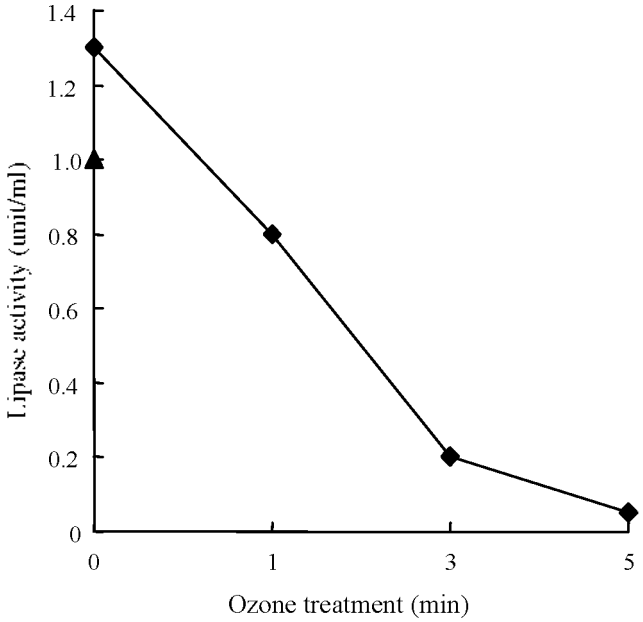

Figure 2. Effect of ozone treatment on lipase activity in freeze-concentrated milk. Ozone concentration was 150 ppm at 7 L/min air flow rate.

min ozone treatment (Table 1). This is accordance to our previous study (Kwak et al., 2002), which has reported that the activity of lactase was reduced to 94.3% after 1 ppm of ozone treatment. Therefore, the present results indicated that 5 min for 150 rpm ozone treatment may be sufficient for inactivation of enzymes and pasteurization of freeze-concentrated milk.

Changes of freeze-concentrated and evaporated milks during storage

Chemical composition : To compare milk composition by concentration processes either the evaporated or the freeze-concentrated processes, the chemical composition was measured as shown in Table 2. No difference was found between two samples in all chemical compositions. Total solid contents were 27.1 and 27.2% in the evaporated and the freeze-concentrated milks, respectively. In both milk samples, the moisture content was 72.9%, and the fat content was 8.5-8.6%.

Even though much progress has been made in the development of freeze concentration technology over the past few decades, only 10-17% skim milk was established by Zhang and Hartel (1995). Therefore, 27% of concentration rate in the present study may be considered as the mostly progressed technique for freeze concentration process.

Microbial counts : The change of total microbial counts of the freeze-concentrated milk, which was pasteurized by ozone treatment and stored at 4°C for 18 day was compared with that of the evaporated milk and is shown in Table 3. During storage, the mean of total microbial counts were not significantly different in all periods. At 0 day, the mean count was 4.0×10^3 CFU/ml and reached to $1.8\text{-}2.0\times10^4$ CFU/ml at 12 day storage in both the evaporated and freeze-concentrated milks. However, microorganism counts were over 10^5 CFU/ml at 18 day storage, which is not generally acceptable milk quality in the market. The present study indicated that 12 day storage could be a maximum limit without aseptic sterilization in evaporated milk, and freeze-concentrated milk with ozone treatment.

TBA test : Heat treatment applied to the evaporated milk is known to cause fat oxidation, resulting in an unpleasant odor and flavor. This potential of oxidized off-flavor and taste could be the main problem in the evaporation process, which needed to be overcome in the concentrated milk production. Therefore, we compared the change of TBA value of the freeze-concentrated milk with that of the evaporated milk during 18 day storage as shown in Figure 3.

Table 2. Composition of freeze-concentrated and evaporated milks (%)[1]

Treatment	Moisture	Protein	Fat	Lactose	Ash	Total solid
Evaporated[2]	72.9±5.1[a]	7.8±1.0[a]	8.5±0.5[a]	9.1±0.9[a]	1.7±0.1[a]	27.1±2.1[a]
Freeze-concentrated[3]	72.8±3.9[a]	7.8±0.9[a]	8.6±0.9[a]	9.0±0.5[a]	1.8±0.2[a]	27.2±1.9[a]

[1] Means with column by the same letter are not significantly different ($p>0.05$).
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

Table 3. Microbial counts in freeze-concentrated and evaporated milks during storage at 4°C for 18 days[1]

Treatment	Storage period (day)			
	0	6	12	18
	CFU/ml			
Evaporated[2]	$4.0\times10^3\pm0.2^b$	$4.6\times10^3\pm0.1^b$	$2.0\times10^4\pm0.2^b$	$2.5\times10^5\pm0.1^{ab}$
Freeze-concentrated[3]	$4.0\times10^3\pm0.1^b$	$5.2\times10^3\pm0.1^b$	$1.8\times10^4\pm0.1^b$	$2.1\times10^5\pm0.2^{ab}$

[1] Means by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min.
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

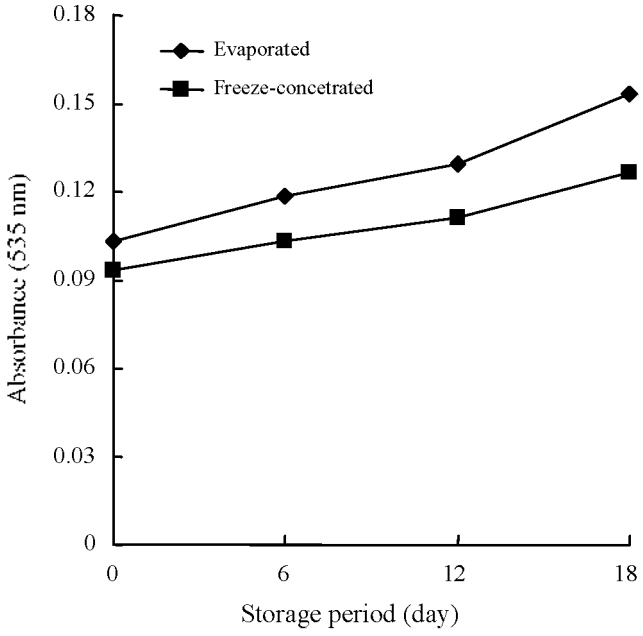

Figure 3. Changes of thiobarbituric acid values (TBA) of freeze-concentrated and evaporated milks during storage at 4°C for 18 days. Ozone concentration was 150 ppm at 7 L/min air flow rate.

TBA absorbance increased with storage in both samples, and especially, the sharp increase was found in the evaporated milk sample. The change of TBA value in evaporated milk was from 0.103 to 0.153, whereas 0.086 to 0.111 in freeze-concentrated milk at 0 and 18 day storage, respectively. Therefore, these results indicated that the evaporated milk which was heat-treated may be susceptible to lipid oxidation rather than the freeze-concentrated milk.

It is known that the velocity of milk fat autocatalytic oxidation grows by the increasing the concentration rate of milk (Caric, 1994b), which explains the reason that the increase of TBA value increase in concentrated milks in this study. Oxidative changes of milk fat may happen during storage of concentrated and dried dairy products containing fat. These changes are attributed to the presence of a high content of unsaturated fatty acids in milk fat. Oxidative reaction of milk fat occurs in the presence of oxygen and it is catalyzed by light and metal ions, such as copper and iron. Based on above information, to protect from the fat oxidation, packaging the concentrated milk in a partial vacuum could be the most important process and it is achieved by partial replacement of oxygen with an inert gas such as nitrogen (Caric, 1994c).

Color : Table 4 compared the color value between two concentrated milk samples during storage. L- and a-values were not significantly different between two samples except for 18 day storage. Compared with other color values, b-value was significantly different between two samples in all periods of storage ($p<0.05$).

Generally, this phenomenon of color change is considered to be originated in the consequence of lactose caramelization and Maillard's reaction due to thermal treatment in the evaporated process. This lactose denaturation in milk causes browning and a reaction product with bitter, unpleasant and burned taste in insufficiently controlled thermal processing (Caric, 1994d). Previous reports have shown that consumers have the highest appeal for fluid milks with visual properties of whole milk (Owens et al., 2001). From our experimental results, the process of freeze-concentration induced less discoloration of milk, which can preserve the similar appearance to non-thermal treated fresh milk. Therefore, freeze-concentration and ozone treatment may have some advantages in color appearance of milk rather than conventional thermal concentration process.

Production of short-chain free fatty acids (FFAs) : The production of short-chain FFAs in concentrated milks during storage is shown in Table 5. When the freeze-concentrated milk was compared with the evaporated milk, no difference was found all individual FFAs except for C_{10} at 0 and 6 day storage ($p>0.05$).

During storage, amounts of short-chain FFAs in both the evaporated and the freeze-concentrated milks showed a slight increase during storage period. At initial day, total production of FFAs was 20.37 and 17.42 ppm, and after 18 day storage, those reached 30.00 and 27.14 ppm, respectively. Therefore, the present study indicated that

Table 4. Color changes in freeze-concentrated an evaporated milks during storage at 4°C for 18 days[1]

Storage period (day)	Treatment	L-value	a-value	b-value
0	Evaporated[2]	4.08 ± 0.2^{ab}	0.90 ± 0.02^{b}	4.04 ± 0.2^{a}
	Freeze-concentrated[3]	3.96 ± 0.3^{ab}	1.28 ± 0.06^{ab}	3.31 ± 0.1^{b}
6	Evaporated	3.90 ± 0.2^{ab}	0.94 ± 0.03^{b}	4.13 ± 0.2^{a}
	Freeze-concentrated	3.92 ± 0.4^{ab}	1.14 ± 0.08^{ab}	3.45 ± 0.1^{b}
12	Evaporated	4.08 ± 0.2^{b}	1.01 ± 0.03^{b}	4.12 ± 0.2^{a}
	Freeze-concentrated	3.68 ± 0.2^{b}	1.33 ± 0.02^{ab}	3.42 ± 0.1^{b}
18	Evaporated	4.69 ± 0.2^{a}	0.90 ± 0.02^{b}	4.15 ± 0.2^{a}
	Freeze-concentrated	3.60 ± 0.1^{b}	1.68 ± 0.06^{a}	3.42 ± 0.1^{b}

[1] Means with column by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min.

[2] 27% total solid evaporated milk.

[3] 27% total solid freeze-concentrated milk.

Table 5. Concentration of short-chain free fatty acids in freeze-concentrated and evaporated milks during storage at 4°C for 18 days[1]

Storage period (day)	Treatment	Short-chain FFA concentration (ppm)				
		C_4	C_6	C_8	C_{10}	Total
0	Evaporated[2]	7.99±0.7^{ab}	3.86±0.2^{b}	3.16±0.2^{bc}	5.36±0.4^{b}	20.37±1.2^{ab}
	Freeze-concentrated[3]	6.55±0.5^{b}	3.36±0.3^{b}	2.87±0.2^{c}	4.64±0.3^{c}	17.42±1.2^{b}
6	Evaporated	8.17±0.2^{ab}	3.57±0.2^{b}	3.25±0.2^{bc}	5.52±0.2^{b}	20.51±1.4^{ab}
	Freeze-concentrated	6.73±0.3^{b}	3.81±0.2^{b}	3.14±0.1^{bc}	4.21±0.2^{c}	17.89±1.1^{b}
12	Evaporated	8.99±0.2^{ab}	4.40±0.2^{ab}	3.57±0.1^{b}	6.12±0.6^{ab}	23.08±1.3^{ab}
	Freeze-concentrated	7.37±0.2^{ab}	4.32±0.2^{ab}	3.50±0.2^{b}	5.45±0.2^{b}	20.64±1.7^{ab}
18	Evaporated	11.15±0.6^{a}	5.95±0.2^{a}	5.05±0.2^{a}	7.85±0.1^{a}	30.00±2.6^{a}
	Freeze-concentrated	9.87±0.7^{ab}	5.86±0.3^{a}	4.85±0.3^{a}	6.56±0.2^{a}	27.14±2.1^{a}

[1] Means with column by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min.
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

Table 6. Concentration of water-soluble vitamins in freeze-concentrated and evaporated milks during storage at 4°C for 18 days[1]

Storage period (day)	Treatment[2]	Water-soluble vitamins (ppm)			
		L-ascorbic acid	Niacin	Thiamin	Riboflavin
0	Evaporated[2]	1.20±0.1^{b}	0.88±0.02^{b}	0.14±0.01^{ab}	0.51±0.00^{b}
	Freeze-concentrated[3]	2.65±0.2^{a}	1.17±0.09^{a}	0.18±0.01^{a}	1.27±0.09^{a}
6	Evaporated	1.01±0.1^{b}	0.87±0.05^{b}	0.12±0.01^{b}	0.45±0.02^{b}
	Freeze-concentrated	2.47±0.2^{a}	1.15±0.03^{a}	0.17±0.01^{b}	1.25±0.02^{a}
12	Evaporated	0.87±0.1^{bc}	0.82±0.02^{b}	0.10±0.01^{b}	0.41±0.02^{b}
	Freeze-concentrated	2.21±0.1^{ab}	1.12±0.05^{a}	0.14±0.01^{ab}	1.22±0.05^{a}
18	Evaporated	0.67±0.06^{c}	0.76±0.02^{b}	0.07±0.00^{c}	0.36±0.02^{b}
	Freeze-concentrated	1.92±0.1^{ab}	1.08±0.03^{a}	0.09±0.00^{bc}	1.18±0.03^{a}

[1] Means with column by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min.
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

FFAs production may be increased by storage period, however, no difference was attributed by the concentration process.

Hydrolytic changes of milk fat in this product are rare, since lipase, which catalyzes lipolysis (i.e. the release of free fatty acids) is activated by proper heat treatment during processing. Therefore, the greater amount of FFAs in the evaporated milk was expected since heat treatment is necessary for the increase in lipase activity (Caric, 1994c).

Vitamins : The concentrations of most water-soluble vitamins were significantly lower in the evaporated milk than those in freeze-concentrated milks ($p<0.05$) (Table 6). Within the sample, the concentrations of all water-soluble vitamins decreased during storage periods.

Since the concentrations were dramatically lower in two concentrated milks, here those results are described. When two concentrated milk samples were compared, L-ascorbic acid and riboflavin concentrations in the evaporated milk were markedly lower than those in the freeze-concentrated milk at every storage period.

The nutritive value of dairy products is based on that of milk itself and is affected by the processing technology used. However, the modern technology used in processing concentrated and dried products (vacuum evaporation, spray drying, multi-stage drying) is known to produce minimal undesirable changes in the milk components. Practically, all milk components after evaporation and drying by such methods are concentrated without any negative changes in value. Still, lysine and lactose undergo minor changes due to Millard reactions, but this has no major impact on the nutritional value of the product if the product is not allowed to absorb water.

Production of free amino acids (FAA) : There was no difference in all individual and total amino acids between the evaporated and freeze-concentrated milks (Table 7). The total FAA amount was steadily increased during 18 day storage. In the freeze-concentrated milk, the change was 759.7 to 1.382.5 μmol/L at 0 and 18 day storage, respectively.

High total solid concentration has a negative influence on the stability of the milk protein system, especially if the system is exposed to the effect of high temperature, as it is in processing. If milk has increased acidity as well, it may completely destabilize and coagulate during sterilization (Caric, 1994b). The higher amount of individual amino acids may be resulted from both unstable protein system and the increased acidity by the effect of heat treatment in evaporated milk.

Table 7. Concentration of free amino acids in freeze-concentrated and evaporated milks during storage at 4°C for 18 days[1]

Storage period (day)	Treatment	Free amino acids (μmol/L)															
		Asp	Glu	Ser	Asn	Thr	Ala	Arg	Tyr	Val	Met	Ile	Leu	Phe	Trp	Lys	Total
0	Evaporated[2]	46.8^{b}	142^{b}	50.4^{b}	12.5^{b}	39.5^{c}	22.4^{a}	32.9^{ab}	139^{b}	122^{b}	17.3^{b}	34.4^{a}	21.9^{b}	29.9^{b}	16.8^{a}	21.7^{bc}	749.3^{bc}
	Freeze-concentrated[3]	48.3^{b}	145^{b}	48.5^{b}	11.2^{b}	34.5^{c}	21.9^{a}	33.3^{ab}	140^{b}	126^{b}	19.2^{b}	37.4^{a}	24.2^{b}	29.1^{b}	17.4^{a}	23.7^{bc}	759.7^{bc}
6	Evaporated	55.1^{ab}	221^{ab}	55.8^{ab}	12.3^{b}	36.5^{c}	24.6^{a}	36.4^{ab}	167^{ab}	151^{ab}	19.8^{b}	37.3^{a}	27.2^{ab}	37.7^{ab}	14.4^{ab}	25.4^{bc}	921.5^{b}
	Freeze-concentrated	58.2^{ab}	211^{ab}	57.7^{ab}	12.8^{b}	38.6^{c}	22.6^{a}	38.7^{a}	179^{ab}	145^{ab}	20.6^{b}	39.7^{a}	26.1^{ab}	37.2^{ab}	16.6^{a}	28.0^{bc}	931.8^{b}
12	Evaporated	68.7^{a}	252^{a}	63.3^{a}	18.4^{ab}	57.8^{b}	26.2^{a}	45.1^{a}	202^{a}	169^{a}	25.7^{ab}	40.2^{a}	27.3^{ab}	38.4^{ab}	17.7^{a}	46.5^{b}	1,098.3^{a}
	Freeze-concentrated	62.8^{a}	259^{a}	65.2^{a}	19.6^{ab}	60.7^{b}	25.2^{a}	47.3^{a}	210^{a}	172^{a}	29.3^{a}	41.5^{a}	29.1^{ab}	43.7^{a}	18.5^{a}	48.2^{b}	1,132.1^{a}
18	Evaporated	73.6^{a}	302^{a}	81.2^{a}	24.7^{a}	82.9^{a}	31.7^{a}	48.5^{a}	271^{a}	205^{a}	32.9^{a}	44.5^{a}	45.3^{a}	45.4^{a}	12.9^{ab}	74.1^{a}	1,375.7^{a}
	Freeze-concentrated	71.9^{a}	298^{a}	78.1^{a}	23.2^{a}	86.4^{a}	29.3^{a}	48.2^{a}	279^{a}	201^{a}	36.8^{a}	43.1^{a}	44.2^{a}	49.4^{a}	18.5^{a}	75.4^{a}	1,382.5^{a}

[1] Means with column by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min.
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

Table 8. Sensory characteristics of freeze-concentrated and evaporated milks during storage at 4°C for 18 days[1]

Storage period (day)	Treatment	Sensory description							
		Cooked flavor	Oxidized flavor	Cheesy flavor	Fat-taste	Oxidized Taste	Off-taste	Yellowish color	Overall acceptability
0	Evaporated[2]	5.4±0.1^{a}	4.5±0.2^{b}	5.0±0.1^{ab}	5.8±0.5^{a}	4.5±0.1^{b}	5.0±0.3^{b}	5.7±0.3^{a}	4.8±0.2^{a}
	Freeze-concentrated[3]	4.8±0.1^{b}	4.7±0.3^{b}	4.8±0.1^{b}	5.3±0.4^{b}	4.7±0.2^{b}	5.3±0.4^{ab}	5.5±0.4^{a}	5.0±0.3^{a}
6	Evaporated	5.4±0.2^{a}	4.7±0.1^{b}	4.8±0.2^{b}	5.7±0.3^{a}	5.3±0.4^{ab}	5.5±0.2^{ab}	5.5±0.5^{a}	4.7±0.4^{a}
	Freeze-concentrated	4.8±0.2^{b}	4.7±0.3^{b}	4.8±0.3^{b}	5.8±0.2^{a}	5.5±0.1^{ab}	5.5±0.5^{ab}	5.2±0.2^{b}	4.8±0.3^{a}
12	Evaporated	5.7±0.1^{a}	5.3±0.1^{ab}	5.7±0.4^{a}	5.8±0.3^{a}	6.5±0.3^{a}	6.0±0.6^{a}	5.7±0.4^{a}	3.8±0.2^{ab}
	Freeze-concentrated	4.5±0.1^{b}	5.5±0.4^{ab}	5.3±0.2^{ab}	6.0±0.4^{a}	6.3±0.6^{a}	6.0±0.3^{a}	5.5±0.5^{a}	4.0±0.3^{ab}
18	Evaporated	5.9±0.3^{a}	6.2±0.3^{a}	5.7±0.3^{a}	5.8±0.3^{a}	6.8±0.4^{a}	6.5±0.2^{a}	5.7±0.3^{a}	2.5±0.2^{b}
	Freeze-concentrated	4.5±0.4^{b}	6.5±0.5^{a}	5.3±0.4^{ab}	6.2±0.2^{a}	6.8±0.3^{a}	6.5±0.5^{a}	5.2±0.1^{b}	2.7±0.3^{b}

[1] Means with column by the same letter are not significantly different ($p>0.05$). Both the evaporated and the freeze-concentrated milks were treated with 150 ppm ozone concentration at 7 L/min air flow rate for 5 min. The scale of cooked flavor, oxidized flavor, cheesy flavor, fat-taste, oxidized-taste, off-taste and color scores: 1 = very slight, 2 = slight, 3 = slight-moderate, 4 = moderate, 5 = moderate-strong, 6 = strong, 7 = very strong. The scale of overall acceptability score: 1 = dislike extremely, 4 = neither like nor dislike, 7 = like extremely.
[2] 27% total solid evaporated milk.
[3] 27% total solid freeze-concentrated milk.

Sensory evaluation : The sensory attributes of both concentrated milks were compared in Table 8. Cooked flavor was significantly higher in the evaporated milk than in the freeze-concentrated milk at every storage periods, as expected. There was no difference in oxidized flavor and taste between the evaporated and the freeze-concentrated milks, however it increased with storage period. The overall quality was highly affected by both the evaporation and freeze-concentration processes. Scores in both samples were about 5, which were in the middle "like or dislike", therefore, concentration process may result in defects in overall acceptability of milk. Overall acceptability was significantly decreased with the storage periods, whose value was about 2.5.

Cooked flavor develops when hydrogen sulfide (H_2S) is released by heat from the amino acids of milk protein that contain sulfur such as cystein. The higher the temperature and longer the time of heating, the greater cooked flavor. Also, there is less damage, equivalent heating to flavor of cream products than to skim milk (Campbell and Marshall, 1975b).

The effects of heat treatment on the components of milk (proteins, lipids, lactose and minerals) are very important for the character of final products, since they undergo modifications that affect the sensory and nutritional quality of milk. The effects of the wide range of technological processes used in the dairy industry may be evaluated by determining several chemical compounds specifically related to such processes, either through degradation of original milk components or as a result of reactions at the high temperatures used.

Based on these results, the evaporated milk showed the stronger cooked flavor compared with the freeze-concentrated milk indicating that heat treatment may

attribute those changes in sensory analysis. In addition, the storage for 18 day may not be acceptable for freeze-concentrated milks with ozone treatment.

CONCLUSION

This study was designed to compare property changes of freeze-concentrated with evaporated milks during storage. For the pasteurization, ozone was treated with the freeze-concentrated milk. The water content was decreased up to 72.8% with newly developed freeze concentration process. With 150 rpm ozone treatment for 5 min 99% of microflora was eliminated, and the activities of protease and lipase decreased 93.31 and 96.15%, respectively. During 12 day storage at 4°C, total bacteria count was maintained below 2.0×10^{4} CFU/ml. Since the evaporated milk was treated by heat, most physicochemical properties were more deteriorated than those in control and freeze-concentrated milk. TBA value and the short-chain fatty acid concentration were significantly higher in evaporated milk than those in freeze-concentrated milk. In sensory evaluation, cooked flavor and color were much higher in the evaporated sample than the freeze-concentrated. Overall acceptability score was significantly lower in both samples after 12 day storage. The present study indicated that the newly developed freeze concentration technique showed the improved physicochemical and sensory properties and little change of most nutrient compounds when compared with the evaporation process. In addition, ozone treatment could be useful to pasteurize the concentrated milk.

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