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SCIENTIFIC PAPER

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EFFECT OF STORAGE PROCESS ON NUTRITIVE PROPERTIES OF PRETERM HUMAN MILK

Article Highlights

- Storage and pasteurization change the lipid and protein properties of preterm human milk
- Lipid content is additionally reduced when milk is pasteurized after freezing
- A fortifier can compensate for deficiencies in preterm milk after storage and pasteurization

Abstract

Freeze storage and pasteurization of human milk are common treatments in milk banks. However, thermal treatment changes milk quality for preterm infants' nutrition. Therefore, this paper aimed to examine preterm human milk's nutritional profile and antioxidant potential after storage, pasteurization, and after supplementation with a fortifier. The effects of storage processes were estimated on the mature preterm milk of 30 breastfeeding women. Total proteins, lipids, and lactose were determined after thermal processing and supplementing mature preterm milk with a fortifier. The ferric-reducing antioxidant potential method and lipid peroxidation inhibition assay determined the antioxidant capacity. Protein concentration decreased after frozen storage and pasteurization ($p<0.05$). Pasteurization further reduced the lipid concentration after freezing. The ferric-reducing antioxidant potential decreased after thermal treatments ($p<0.05$). Supplementing mature milk with a fortifier increased the concentration of proteins, lipids, and lactose. Our findings demonstrated that storage and pasteurization processes affect preterm human milk's basic nutritional composition and antioxidant capacity. To ensure adequate nutrition for preterm infants with preterm human milk, supplementation, especially with high concentrations of proteins and lipids, is necessary after thermal treatments.

Keywords: preterm human milk, pasteurization, freeze storage.

The use of human milk in feeding premature babies is very important and in accordance with the WHO recommendations for using human milk or pasteurized donor milk in feeding early premature babies, which begins in neonatal units [1]. Feeding premature infants with the breast milk of mothers of premature infants are associated with numerous beneficial effects, and it is a key component in the strategy of enteral nutrition for premature babies. In addition to nutritional components, human milk contains biologically important ingredients, such as immunoglobulin, enzymes, immune factors, growth factors, and hormones that can affect growth and development [2,3]. Furthermore, when the mother is separated from the child, she can still secrete milk and store it for later feeding a sick or premature infant. Preterm infants spend the first weeks of their lives in special neonatal units, where human milk nutrition is very important and which supports the storage of milk in milk banks [4,5]. However, storage methods, parameters, and heating processes can affect the quality of human milk's nutritional components [6].

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The antioxidant concentration is a very important factor for determining the quality of the milk used in the nutrition of premature infants [7]. Premature infants are exposed to oxidative stress after birth, and it is considered that supplementation with enzymatic and non-enzymatic antioxidants can prevent the development of diseases, affect the immune system of the newborn and increase neonatal vitality [8]. In addition to enzymatic systems, many small molecules participate in the prevention of oxidative stress, such as carotene, lipoic acid, enzyme inhibitors, antioxidants, enzyme cofactors, and transition metal chelators [9,10].

Lipid peroxidation is an indicator of oxidative stress in cells and tissues. Peroxides of polyunsaturated fatty acids generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) after decomposition, and MDA and HAE levels can be used as lipid peroxidation indicators [11].

Ferric reducing antioxidant potential (FRAP) indicates the levels of antioxidants that act as reductants in redox methods. FRAP depends on the degree of conjugation and the number of hydroxyl groups in the milk [12].

However, preterm infants' intake of macronutrients can be inadequate due to differences in the individual components of human milk. In fact, a premature infant diet consisting exclusively of preterm breast milk over a longer period can be associated with lower growth and progression and the development of nutritional deficit, compared to an infant fed with milk supplemented with fortifier or infant formula for prematurely born children [2,3].

Milk fortifier for human milk supplementation has been introduced to support breastfeeding in premature infants' diets. Fortifiers increase the nutritional value of the milk by adding proteins, vitamins, minerals, and energy, which helps cater to the high nutritional needs of these fast-growing babies [13,14]. Since the composition of human milk can be altered by storage and pasteurization, these are important factors for milk management by milk banks. The nutritional management of high-risk infants using human milk requires individualized, adaptive, or targeted strategies for fortification based on the measurement of milk composition and growth monitoring [15].

This study aimed to examine milk quality from lactating mothers of preterm infants after Holder pasteurization and storage at -20 °C. The nutritional components (protein, fat, and carbohydrate concentrations) and antioxidant capacities related to nutrient activity were determined for that purpose.

EXPERIMENTAL

Study design

Thirty healthy mothers of preterm infants were included in the study conducted at the milk bank of the Institute of Neonatology, Belgrade, Serbia, approved by Institutional Ethics Committee N82401/4 (April 18, 2014). Informed consent was obtained from all participants. Exclusion factors for the study were smoking, and medicaments contraindicated for breastfeeding. The mean age of participants was 27 ± 3 years, and their mean body mass index, mean infant birth weight, and mean gestation week were 23 ± 3.6, 1560 ± 418 g, and 31 ± 2, respectively. Milk samples were collected during different lactation phases: colostrums (4 days postpartum) and transient and mature milk (14 and 21 days after delivery, respectively). The effects of storage and pasteurization were studied in mature milk only. Mature milk samples were examined for each treatment 1: Mature milk, as freshly expressed, homogenized mature milk; 2: Pasteurized milk, mature milk after Holder pasteurization (62.5 °C for 30 min); 3: Milk after seven days storage at -20 °C; 4: Milk after seven days storage at -20 °C, and pasteurized; 5: Milk after 30 days storage at -20 °C; 6: Milk after 30 days storage at -20 °C, and pasteurized. Before measurement, all milk samples were homogenized by an Ultrasonic homogenizer Sonopuls 2000.2 (Bandelin, Berlin, Germany). Fresh milk samples were analyzed immediately after collection, while storage, freezing, and pasteurization effects were examined after thawing and homogenization.

Multicomponent fortifier (FF), produced in Serbia and designed for preterm infants, was used to supplement human milk (5 g FF was added to 100 ml human milk). The fortifier had the following declared basic nutritional information: proteins, 20.8%; carbohydrates, 64.3%; and fat, 4.4%, with ingredients: maltodextrin, prolactal, mineral and vitamin premix, micronutrient premix, refined edible soybean oil, and lecithin [16].

Analyses of nutritional parameters in human milk samples

Human milk samples were analyzed for total protein concentration using the Bradford method [17]. Total fat concentration in milk samples was determined by the Weibull-Berntrop gravimetric method (ISO 8262-1|IDF 124-1:2005) for infant foods [18]. Lactose concentration was determined using Megazyme, Ireland (K-LACGAR), with the assay performed according to the guidelines supplied by the producer [19]. The total carbohydrate concentration was assessed based on the difference between protein,

fat, water, and ash calculated values. The energy content was also calculated, expressed as kJ/100 mL or kcal/100 mL [20]. All nutritional parameters, protein, fat, lactose, and total carbohydrates were expressed as g/100 mL of milk sample. Ferric reducing antioxidant potential (FRAP), as a direct method for measuring the total antioxidant power of biological fluids, was assessed according to Benzie and Strain. The results were expressed as FRAP values (μmol Fe^{2+}/L) [21]. Lipid peroxidation inhibition assay (ILP) was used as an indicator of oxidative stress in milk samples for screening and monitoring lipid peroxidation and non-enzymatic antioxidant activity in milk samples [22]. Malondialdehyde formed by the dissolution of unsaturated fatty acids serves as an index for determining the strength of the peroxidation reaction. It was detected by the thiobarbituric acid assay; the product was determined at 535 nm and expressed in μmol MDA/L.

Statistical analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS software package, version 25.0; SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). The normality of distributions was tested using the Koglomorov-Smirnov test. If the data were normally distributed, RM ANOVA was used. Non-parametric data were analyzed using the Friedman test. The Pearson correlation coefficient was used to examine the relationship between two observed parameters. All reported p values were two-sided; p values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The nutritional profile of human milk in different phases of lactation and after storage

The nutritional profiles of human milk in different phases of lactation are presented in Figure 1A. The mean protein concentration in the human milk was significantly different ($p < 0.05$) in the different lactation stages. Preterm colostrums had a higher protein concentration than transient and mature milk, with maximum mean differences occurring in the first few days after birth ($p < 0.05$). There were no statistically significant differences in protein concentration between transient and mature milk. Lipid concentrations of colostrums were statistically different from those of transient and mature human milk samples, while there was no difference between transient and mature milk lipid concentrations ($p > 0.05$). Total carbohydrates in colostrum were significantly lower than in human milk after 14 and 21 days after birth. There was no statistical difference in total carbohydrate concentration between transient and mature milk ($p = 0.130$), while lactose contents differed during the lactation period ($p < 0.05$). Significant differences ($p < 0.05$) in fat and total carbohydrate concentration were observed between colostrum and transient and mature milk examined directly after the milk was expressed at room temperature. However, the Bonferroni pairwise comparison test indicated no significant difference ($p > 0.05$) between transient and mature human milk at room temperature.

Figure 1B shows changes in protein, fat, lactose, and total carbohydrate concentration after mature milk storage at -20 °C for 7 and 30 days and after pasteurization. Protein content slightly decreased and differed after storage at -20 °C for 7 and 30 days and after pasteurization ($p < 0.05$). The mean lipid concentration in milk after frozen storage and pasteurization was lower than in fresh milk ($p < 0.05$). Pasteurization reduced the lipid concentration of mature milk compared to that of fresh mature milk (6%). Freezing at -20 °C for 7 or 30 days did not statistically affect the lipid concentration of mature milk (3-4%; $p > 0.05$). However, storage by freezing for 7 and 30 days and pasteurization immediately before analysis significantly reduced the mean lipid concentration in milk compared to fresh mature milk and mature milk stored by freezing at -20 °C ($p < 0.05$). Freezing changes the physicochemical properties of human milk components. As milk proteins precipitate, casein micelles destabilize, and protein structure changes. Thawing and homogenization do not entirely decompose protein aggregates or lipid globules, especially when manual homogenization is performed. Holder pasteurization increases the effect of freezing on protein and lipid structure, leading to enzyme activity modifications and increased lipolysis [23]. There was no significant change in the lactose concentration of preterm milk after frozen storage and pasteurization. Freezing and pasteurization preserved the mean carbohydrate concentration in the mature milk, and the mean carbohydrate concentration in the stored mature milk did not differ statistically from that of fresh mature milk. There was no significant change in the lactose concentration of mature milk after freezing (compared with lactose levels in fresh mature milk).

The total energy content of preterm milk increased during lactation phases: colostrum 49.9 kcal/100 mL, transient milk 67.5 kcal/100 mL, and mature milk 69.6 kcal/100 mL (Figure 2a). Pasteurization after 7 or 30 days of storage at -20 °C additionally decreased the energy content of human milk ($p < 0.05$), while only storage at -20 °C did not affect the energy content of preterm milk ($p > 0.05$, Figure 2b).

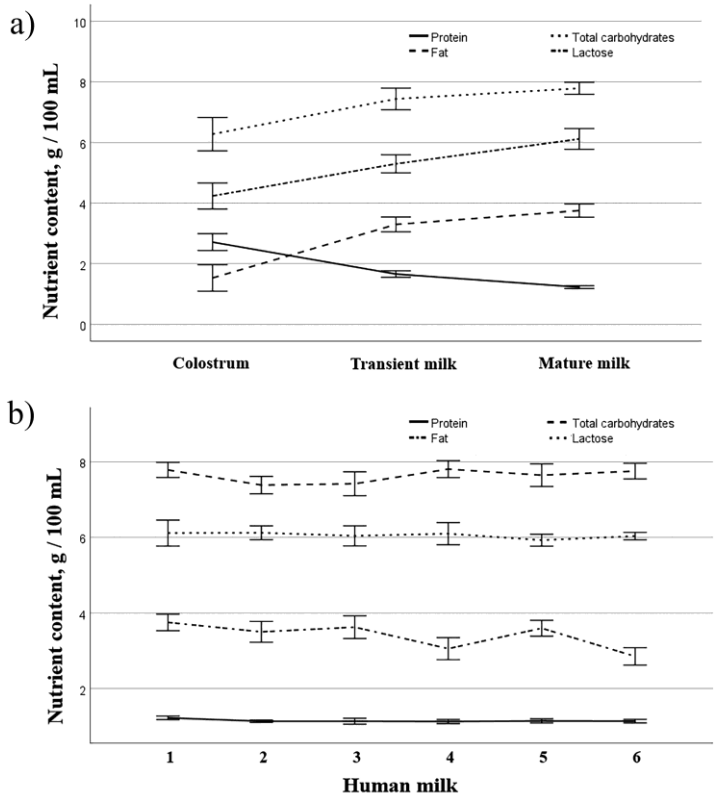

Figure 1. The nutritional profile of human milk in different phases of lactation (A) and after storage and pasteurization treatments (B) A: Colostrum, Transient and Mature milk; B: Human milk samples: 1: Mature milk, 2: Pasteurized milk, 3: Milk after seven days storage at -20 °C, 4: Milk after seven days storage at -20 °C, and pasteurized, 5: Milk after 30 days storage at -20 °C, 6: Milk after 30 days storage at -20 °C, and pasteurized.

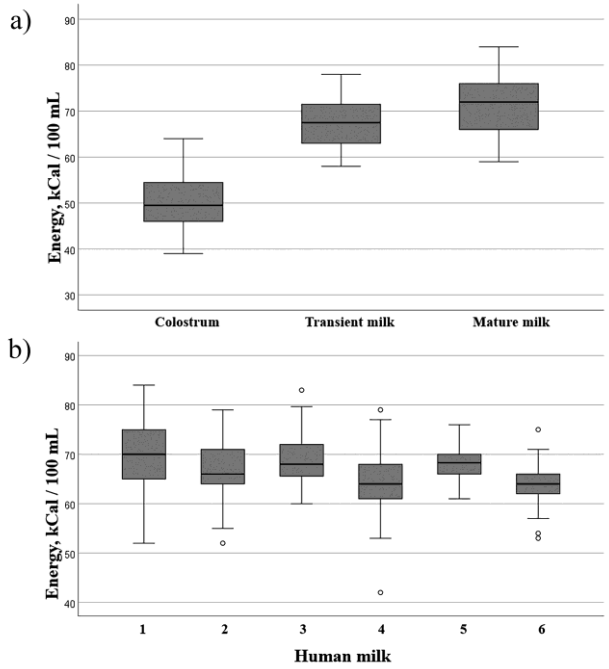

Figure 2. The total energy content of human milk in different phases of lactation (A) and after storage and pasteurization treatments (B) A: Colostrum, Transient and Mature milk; B: Human milk samples: 1: Mature milk, 2: Pasteurized milk, 3: Milk after seven days storage at -20 °C, 4: Milk after seven days storage at -20 °C, and pasteurized, 5: Milk after 30 days storage at -20 °C, 6: Milk after 30 days storage at -20 °C, and pasteurized.

The antioxidant profile of human milk in different phases of lactation and after storage and pasteurization

Figure 3 shows the ILP antioxidant profile of human milk determined by the thiobarbituric acid assay. The concentration of MDA differed slightly according to the lactation phase (p> 0.05), while there was no statistically significant difference due to storage and pasteurization. Fresh mature milk and mature milk after freezing and pasteurization did not show any significant level of lipid peroxidation. The antioxidants in milk likely contributed to the minimal degradation of lipids and proteins. Freezing and pasteurization reduced lipid peroxidation while freezing slightly increased the concentration of MDA but not significantly.

The ferric-reducing antioxidant potential is a biochemical indicator of the antioxidant capacity of human milk in different stages of lactation (Figure 4A). During the lactation period, there was a statistically significant difference (p = 0.029) in FRAP activity between colostrum and transient and mature milk, while there was no difference in FRAP activity between the transient and matured milk (p >0.05). Pasteurization did not affect FRAP compared to raw mature milk. However, the FRAP's antioxidant capacity was significantly reduced after seven days of storage at -20 °C and pasteurization (p <0.05) compared to unprocessed milk. Furthermore, the deleterious effects of pasteurization on FRAP became significant after seven days of storage and pasteurization, while there was no significant difference in FRAP between mature milk and milk after 30 days of storage and pasteurization (Figure 4B).

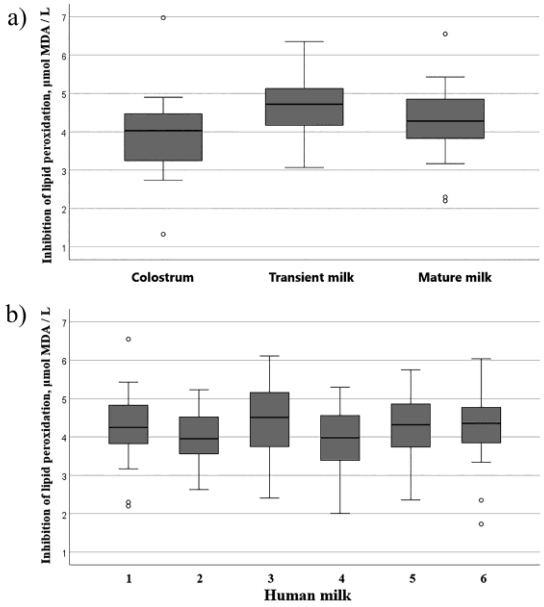

Figure 3. The antioxidant profile of human milk in different phases of lactation (A) and after storage and pasteurization treatments (B) determined by lipid peroxidation inhibition assay (ILP). Human milk samples after storage: 1: Mature milk, 2: Pasteurized milk, 3: Milk after seven days storage at -20 °C, 4: Milk after seven days storage at -20 °C, and pasteurized, 5: Milk after 30 days storage at -20 °C, 6: Milk after 30 days storage at -20 °C, and pasteurized.

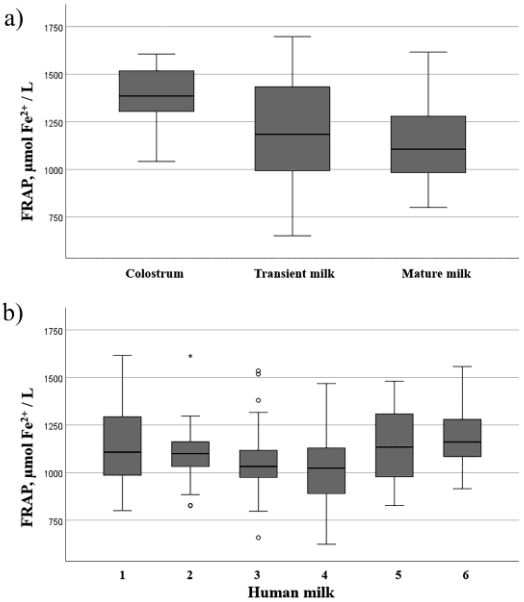

Figure 4. The antioxidant profile of human milk in different phases of lactation (A) and after storage and pasteurization treatments (B) determined by ferric reducing antioxidant potential (FRAP) method. Human milk samples after storage: 1: Mature milk, 2: Pasteurized milk, 3: Milk after seven days storage at -20 °C, 4: Milk after seven days storage at -20 °C, and pasteurized, 5: Milk after 30 days storage at -20 °C, 6: Milk after 30 days storage at -20 °C, and pasteurized.

Table 1 compares the nutritional parameters of the preterm mature milk and the preterm milk supplemented with a fortifier. Supplementing preterm mature milk with the fortifier increased the nutritional value of milk in accordance with the recommendations for preterm infants [15]. Fortifier increased energy by 24% and concentration of protein, lipid, and lactose by 42.0%, 5.2%, and 12.7%, respectively. However, the preterm infant diet in neonatal units was based on the milk stored at -20 °C, which was pasteurized and supplemented with the fortifier immediately before feeding. Storage and pasteurization reduce the antioxidant activity, change the protein and lipid composition and reduce protein and lipid concentrations, so supplementation is necessary. Fortifier increased the nutritional parameters, including the antioxidant activity of non-enzymatic components (by 45-50%; Table 1). In addition, supplementation of preterm milk with fortifier showed an increased FRAP capacity (p< 0.05) and increased concentration of MDA

Table 1. The nutritional profile of preterm mature milk and preterm mature milk supplemented with fortifier.

Nutritional parameters	Preterm mature milk Mean (SD)	Preterm mature milk with fortifier Mean (SD)
Energy, kJ/100 mL	291 (33)	367 (34)
Energy, kcal/100 mL	69.6 (6.7)	87.6 (8.0)
Proteins, g/100 mL	1.23 (0.25)	2.30 (0.13)
Lipids, g/100 mL	3.75 (0.36)	3.95 (0.59)
TC*, g/100 mL	7.78 (0.90)	10.91 (0.53)
Lactose, g/100 mL	6.12 (0.63)	7.51 (0.41)
FRAP, μmol Fe^{2+}/L	1127 (179)	2069.1 (366)
ILP, μmol MDA/L	4.29 (0.89)	10.05 (0.23)

*TC -Total carbohydrates

($p< 0.05$) compared to mature milk.

The composition of human milk during the lactation period varies between mothers and even in individuals during a single day. These multidimensional variations in the composition are due to the mother's adaptation to the infant's needs, the geographical region, and the mode of nutrition [24]. Protein concentration decreased in the preterm milk during lactation stages, and all types of storage affected a slight decrease in the protein concentration of the mature milk. However, there was a difference in the protein concentration in the mature milk samples that underwent different types of storage and pasteurization. Holder's pasteurization deactivated the enzymes and denatured whey proteins, which was in line with previous studies. A previous meta-analysis showed that a high concentration of proteins in the colostrum and early lactation phases resulted from the reduced whey protein concentration, in which lactalbumin, IgA, lactoferrin, and lysosomes were found [25]. The mean lipid concentration of preterm milk increased during lactation, while after freezing, it was lower due to the change of the lipid membrane structure because the fat adheres to the walls of the bottle, as well as due to the inactivation of lipase in the milk, in accordance with the previous studies [26,27].

Preterm milk behaves differently after storage and freezing in relation to full-term milk due to its chemical composition, stability of components, and factors that affect the composition. Therefore, it is subject to different transformations in relation to full-term milk. Preterm infants require an energy-rich diet compared to full-term infants, so human milk is mostly supplemented with fortifiers. The storage process influences the nutritional properties of human milk. This study compared the major nutrient contents in preterm mature milk and preterm mature milk after fortification with a multicomponent fortifier to evaluate the optimization of the nutrient quality of human milk. In addition to nutritional values, the quality of human milk also depends on its antioxidant capacity. The antioxidant capacity of human milk was the greatest in the first weeks of lactation. It was associated with an increased level of oxidative stress that occurs in the mother's body [27,28]. Therefore, total antioxidant capacity measured in the milk samples during lactation and after storage showed the protective role of human milk associated with non-enzymatic antioxidants. Freezing and pasteurizing preterm milk decreased ferric-reducing antioxidant potential and did not affect the lipid peroxidation process. Effects on nutritional and antioxidant properties of milk caused by pasteurization and freezing can be compensated for by supplementing the milk with a fortifier. Fortification of preterm milk should be based on individual milk analysis after thermal treatments.

CONCLUSION

The breast milk given by mothers to preterm infants provides important nutritional components for infant growth and protects against the effects of free radicals, reactive oxygen species, and oxidative stress. Fresh breast milk, especially colostrum, has the required quality and antioxidant properties. When fresh human milk is unavailable, freezing retains human milk's nutritional and antioxidant properties. Freezing is a better storage option than freezing, followed by pasteurization after thawing, especially for preserving the lipid content. Although freezing followed by pasteurization procedures are common in milk banks, they negatively affect the quality of the milk. Therefore, in milk banks, individual milk supplementation with quality fortifiers should provide adequate nutritional components, especially a greater lipid content and a higher concentration of antioxidant components, both of which are required for normal infant development.

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NAUČNI RAD

UTICAJ PROCESA SKLADIŠTENJA NA NUTRITIVNA SVOJSTVA MLEKA MAJKI PREVREMENO ROĐENE DECE

Zamrzavanje i pasterizacija humanog mleka su uobičajeni tretmani u bankama mleka. Termička obrada menja kvalitet mleka za ishranu prevremeno rođene dece, stoga je značaj ovog rada da istraži nutritivni sastav i antioksidativni potencijal mleka majki prevremeno rođene dece nakon skladištenja i pasterizacije i nakon suplementacije obogaćivačem majčinog mleka. Efekti procesa skladištenja su ispitivani na zrelom mleku 30 žena koje su se prevremeno porodile. Nakon termičke obrade i suplementacije majčinog mleka obogaćivačem ispitivan je sadržaj ukupnih proteina, lipida i laktoze. Antioksidativni kapacitet je određen metodom baziranom na reakciji antioksidanasa sa Fe (III)-kompleksom i metodom inhibicije lipidne peroksidacije. Nakon skladištenja zamrzavanjem i pasterizacije opada koncentracija proteina ($p< 0.05$). Pasterizacija nakon skladištenja zamrzavanjem dodatno smanjuje koncentraciju lipida. Antioksidativni potencijal, određen metodom baziranom na reakciji antioksidanasa sa Fe (III)-kompleksom, smanjuje se nakon termičke obrade ($p< 0.05$). Suplementacija zrelog mleka obogaćivačem povećava koncentraciju proteina, lipida i laktoze. Naši rezultati su pokazali da procesi skladištenja i pasterizacije utiču na osnovni nutritivni sastav i antioksidativni kapacitet mleka majki prevremeno rođene dece. Da bi se obezbedila adekvatna ishrana prevremeno rođene dece, neophodna je suplementacija mleka, posebno visokim koncentracijama proteina i lipida, nakon termičke obrade.

Ključne reči: mleko majki prevremeno rođene dece, pasterizacija, skladištenje zamrzavanjem.