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Original Research Article

The effect of Storage conditions on nutritional quality of donor human milk in milk bank practice

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Abstract

Background: The main function of milk banks is to serve as repositories of donated milk so it is available when needed. The concentrations of macronutrients in human milk can be influenced by various processes, such as storage and freezing. The objective of present study was to assess, understand changes in major nutrients, role of antioxidant and free radical generation after milk bank storage.
Method: In this study the human milk samples were collected and analyzed for protein, lactose, fat and antioxidant as vitamin C and marker of free radical MDA. The breast was cleaned with sterile water before expression and by using automatic electric double pumping (medelalactina) 20 ml of milk sample collected in sterile container. The sample were divided in to three aliquotes, first analyzed immediately as fresh and the second refrigerated at 4^0c and third frozen at -20^0C.
Results: There was no significant change in protein and lactose content in breast milk. The values of fat and ascorbic acid significantly decreased at room temperature and 4^0C in day 2 and day 4 respectively but not at -20^0C. End product of lipid peroxidation MDA significantly increased at room temperature and 4^0C but there was no change at -20^0C.
Conclusion: There were no notable changes observed in all the nutritional contents studied even after four days of storage at -20 degrees Celsius. As a conclusion, the nutritional content of expressed milk is safe while kept at -20 degrees Celsius.
Keywords: Human milk, Milk bank, Nutritional value, Lactose, Ascorbic acid.

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1. Introduction

The composition of breast milk includes bioactive factors required to provide appropriate nutrition for developmental processes and offers simultaneously protective functions for the children as well as the mammary glands during lactation [1-3]. Mother's own milk is the first choice for the feeding and nutrition of preterm and term newborns. When their own mothers' milk is unavailable or not sufficient to satisfy the requirements of preterm babies, donor human milk (DHM) is the best alternative for these vulnerable infants [4]. However, human milk (HM) is widely recognized as the optimal feeding for newborn infants, especially for preterm infants, because of its various benefits in term of immunological, gastrointestinal and neurodevelopmental functions [5]. Every year, an estimated 15 million babies are born preterm and these numbers are rising [6]. Appropriate nutrition is a critical factor in efforts to improve survival and outcomes of extremely and very preterm babies [7, 8].

The main function of milk banks is to serve as repositories of donated milk so it is available when needed.

Milk banks receive milk from donors, process it, and store it until used. Most commonly milk from multiple donors is pooled, although some banks pool milk only of individual donors (single-donor banks). Usually, milk provided by milk banks has undergone pasteurization. Once pasteurized, milk is placed in small (100-150 mL) containers and is stored frozen for up to 1 year depending on local guidelines [9, 10]. Milk banks generally follow standardized procedures for the collection and handling of donated milk [10, 11]. Moreover, donors are instructed by the milk bank about recommended breast cleaning and breast pumping procedures. The bank provides containers for milk. Pooling of milk from several pumping is often performed. Each container must carry the name, date, and time of expression. The milk remains in the freezer until it is delivered to the bank.

Cold storage of HM is a routine not only in HM bank but also at home and in the hospitals, especially in Neonatal Intensive Care Units. According to the length and on the typology of storage, the milk banking storage process may partly inactivate nutritional and protective value of human milk which is present and protect human infants against disease. The maximum refrigeration time for human milk ranges between 24 h and 8 days, according to the current advices on safe HM storage [12-14]. Therefore, the objective of present study was to assess, understand changes in major nutrients, role of antioxidant and free radical generation after milk bank storage.

2. Method

After obtaining Institutional Ethical Committee approval and volunteer donors consent total 72 matured human milk samples were collected and analyzed for protein, lactose, fat and antioxidant as vitamin C and marker of free radical MDA. The breast was cleaned with sterile water before expression and by using automatic electric double pumping (medelalactina) 20 ml of milk sample collected in sterile steel container in the OPD LTMGH Sion Mumbai. The sample were divided in to three aliquotes, first analyzed immediately as fresh and the second refrigerated at 4^0C and third frozen at -20^0C.

2.1 Total protein estimated by biuret method:

The classic biuret method [15] is based on thereaction of Cu (ll) ions in an alkaline solution with peptidelinkages in proteins, forming a violet-colored compound thatis quantified spectrophotometrically. This method requires0.02 mL of de-fatted milk and is not very sensitive, thedetection limit being 1 gIL. Several substances in humanmilk may interfere to give a falsely high reading [16].

2.2 Lactose by Benedicts quantitative reagent method:

The Benedicts quantitative reagent method was used to estimate the concentration of lactose present in a sample of milk. The estimation was easily achieved with inexpensive equipment and materials using the method given by Smith and Dawson [17].

2.3 Lipid (fat) by Gottfried and Rosenberg Method:

We have used the procedure of Gottfried and Rosenberg for determination of Lipid (Fat). The method used normane; concentrated aqueous potassium hydroxide as the saponifying reagent; and a somewhat higher saponification, oxidation, and condensation temperature. Consequently, the yellow dehydrolutidine derivative is determined in a homogeneous solution having an almost linear absorbance up to 3g/liter [18]

2.4 Ascorbic acid determination by simple colorimetric method:

A convenient colorimetric method was used for the determination of ascorbic acid from the milk sample as described by Woessner et al [19].

2.5 Determination of MDA and thiobarbituric acid reactive substances

A simple and highly sensitive spectrophotometric method was used for the determination of thiobarbituric acid reactive substances (TBARS) and Malondialdehyde (MDA) in milk sample which is developed by Zeb and Ullah [20]

2.6 Statistical Analysis

Allquantitative data are displayed as mean and standard deviation. All data were put into Microsoft Excel and analysed with SPSS 20.00.*p value*<0.05 considered as a statistically significant

3. Result

Table 1 show the no significant change in protein and lactose content in breast milk. The values of fat (Figure 1) and ascorbic acid (Figure 2) significantly decreased at room temperature and 4^0C in day 2 and day 4 respectively but not at -20^0C. End product of lipid peroxidation MDA significantly increased at room temperature and 4^0C but there was no change at -20^0C, (Figure 3).

Table 1: Changes in the lactose, protein, fats, Ascorbic acid and MDA in human milk during storage at various temperature and duration

Breast Milk content	Storage duration	Temperature		
		RT	4ºC	-20⁰C
Protein±SD (g/dL)	Day 0	3.57±0.18	3.57±0.18	3.57±0.18
	Day 2	2.98±0.14	3.49±0.86	3.51±0.88
	Day 4	2.74±0.81	3.42±0.74	3.49±0.80
p-value		*0.089*	*0.069*	*0.091*
Lactose±SD (g/dL)	Day 0	7.18±0.06	7.18±0.06	7.18±0.06
	Day 2	6.71±0.96	6.84±0.94	7.10±0.98
	Day 4	6.21±0.99	6.53±0.83	6.92±0.76
p-value		*0.065*	*0.163*	*0.083*
Fat±SD (g/dL)	Day 0	2.06±0.5	2.06±0.5	2.06±0.5
	Day 2	1.99±0.47	2.01±0.53	2.05±0.49
	Day 4	1.96±0.50	1.98±0.49	2.06±0.53
p-value		*0.045*	*0.021*	*0.075*
Ascorbic acid±SD (mg/dL)	Day 0	4.94±1.29	4.94±1.29	4.94±1.29
	Day 2	3.55±0.76	4.61±1.33	4.89±1.28
	Day 4	3.07±0.79	4.43±1.21	4.80±1.26
p-value		*0.018*	*0.036*	*0.186*
MDA±SD (µmol/L)	Day 0	1.02±0.33	1.02±0.33	1.02±0.33
	Day 2	1.35±0.44	1.26±0.4	1.03±0.31
	Day 4	1.94±0.52	1.83±0.51	1.04±0.8
p-value		*0.049*	*0.041*	*0.135*

Figure 1: Changes in fats in human milk during storage at various temperature and duration

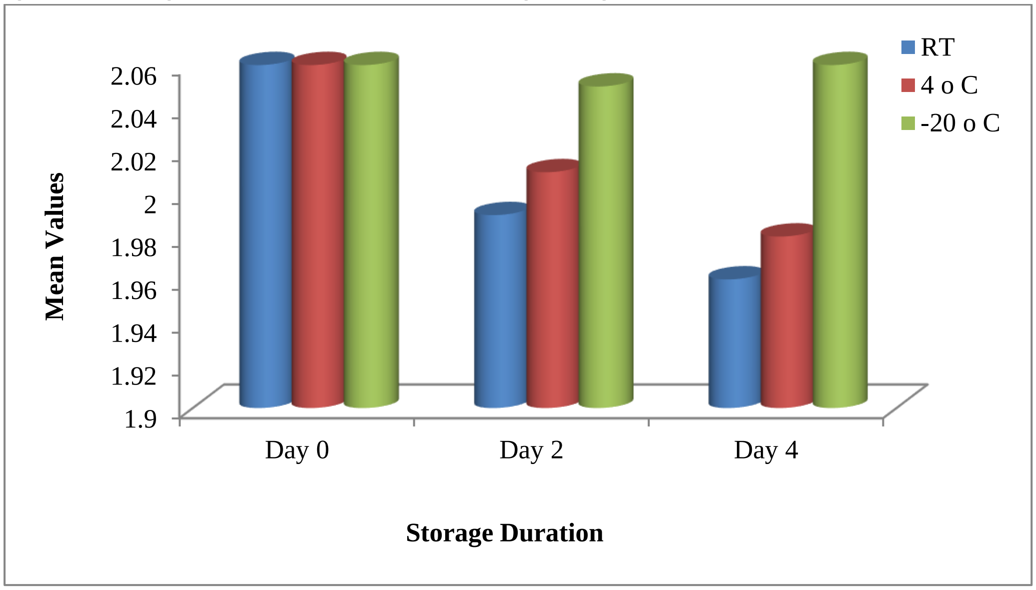


Figure 2: Changes in the Ascorbic acid in human milk during storage at various temperature and duration

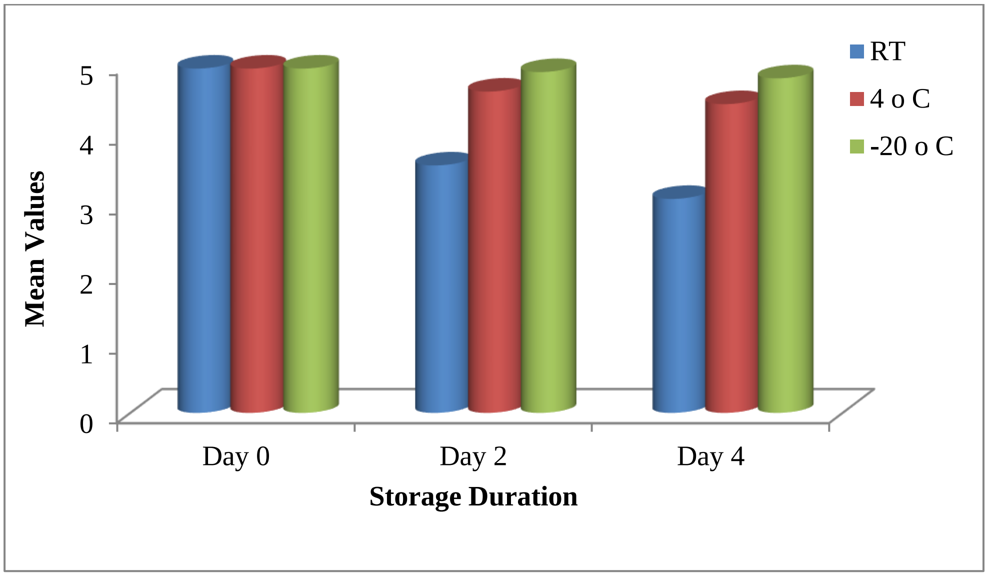
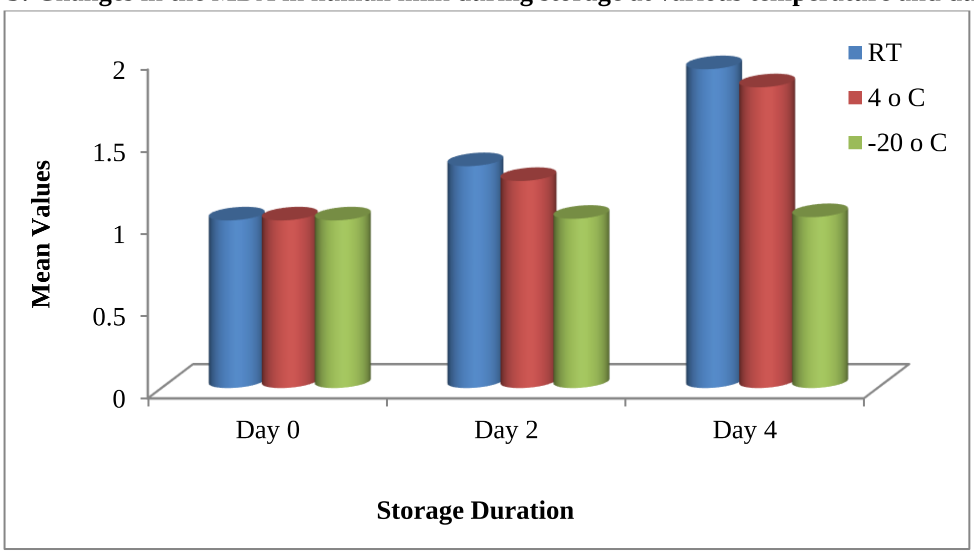


Figure 3: Changes in the MDA in human milk during storage at various temperature and duration



4. Discussion

Human milk bank provides donors' milk to premature infants, many sick babies or mother is unable to provide milk or infants are not able to receive their mothers' milk due to many reasons. The milk banking storage process may partly inactivate nutritional and protective value of human milk which is present and protect human infants against disease. Human milk is a highly complex, and dynamic biological fluid rich in nutritive (e.g., lipids, proteins, carbohydrates, fatty acids, amino acids, minerals, vitamins, trace elements, etc.) and non-nutritive bioactive components (e.g., cells, Ig, cytokines, chemokines, hormones, growth factors, glycans, mucins, etc.). It provides protection against infection and inflammation, and contributes to immune maturation, organ development, microbiota colonization and overall infant health [21, 22]. In this study, we investigated the effect of storage conditions (room temperature, 4^0C and -20^0C) on the composition and functional properties (protein, lactose, fat, ascorbic acid, MDA) of human milk.

Previous studies reported that proteolysis and lipolysis occur in human milk at different temperatures [23-25]. Denaturation of proteins has been noted after freezing (-20°C) and thawing. Hamosh et al [23] reported the storage of human milk is safe at 15 degrees C for 24 hours, whereas at 25 degrees C it is safe for 4 hours. Milk should not be stored at 38 degrees C. In present study it has been shown that no significant changes in concentration of proteins which revealed minimal proteolysis. Minimal proteolysis during storage suggests that milk proteins probably maintain their structure and function during short-term storage.

Furthermore, in current study the variation of storage temperature has insignificant effect on the protein content of the milk. The denaturation of proteins does not occur at various storage temperatures since denaturation occurs only when there is a heat treatment, alcohol, acetone, acids, ultrasonic vibration or ultraviolet radiation. This study was designed to control the factors that cause the denaturation of the protein in the sample. A previous study mentioned that the protein in human breast milk is not denatured at storage temperature of 4ºC for 48hours [8]. The storage of breast milk in the freezer does not have a major influence on theprotein level, so that the milk can be stored safely for 72 hours at a temperature of 4-6^{0}C. The results of our study support the previous study stated that the temperature on different types of storage did not affect the levels of protein.

Milk fat is a highly complex fat, more 200 types of fatty acids have been recognized in it, it is the most common and abundant source of short-chain fatty acids. Specific flavour and taste perspectives of dairy products are mainly due to the short-chain fatty acids. In addition to that, milk fat is also a reasonable source of oleic acid, which is regarded beneficial fatty acid in prevention of dyslipidaemia. Milk fat also contains some concentration of linoleic acid ($C_{18:2}$). Unsaturated fatty acids are usually susceptible to auto-oxidation [26]. Auto-oxidation of unsaturated fatty acids also depends upon the storage temperature. However, fat accounts for approximately 50% of the nonprotein energy in human milk and facilitates the absorption, delivery, and transport of fat-soluble vitamins. The storage of human milk in polyethylene bags results in reduced fat content due to adherence to the inside surface of the bag [27]. Similarly, sterilization of human milk causes a decrease in the fat percentage by enhancing fat adherence to the container surface [28]. Some lipid-soluble nutrients in human milk demonstrate a similar propensity to adhere to the surfaces of containers made of glass and polypropylene [29]. Another study showed that the human milk creamatocrit level remains stable after freezing at -20°C for 28 days, but decreases after two cycles of freezing and thawing [30], and lipolysis could occur after freezing for long periods. In current study, fat significantly decreased at room temperature and 4^{0}C in day 2 and day 4 respectively but not at -20^{0}C. The results revealed that the concentration of fat remains constant when freezing at -20^{0}c till four days. It also suggested that lipolysis may occur after longer period of freezing.

The principal sugar of human milk is the disaccharide lactose. The concentration of lactose in human milk is the least variable of the macronutrients, but higher concentrations of lactose are found in the milk of mothers producing higher quantities of milk [31]. Lactose has been noted as stable after pasteurization, freezing, and thawing in some studies [32, 33], which is consistent with our results that is no significant changes has been observed in lactose concentration during room temperature, 4^{0}C and -20^{0}C at day 0, 2 and 4 respectively.

The L-enantiomer of ascorbic acid, commonly known as vitamin C, is a nutrient whose deficiency causes the disease scorbuticus (scurvy). This essential nutrient is critical to the health and development of infants as it is necessary for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone which develop rapidly during infancy [34]. Ascorbic acid is also a highly effective antioxidant. Even in small amounts it can protect indispensable molecules in the body, such as proteins, lipids, and nucleic acids from damage by free radicals and reactive oxygen species that are generated during normal metabolism and rapid growth. Ascorbic acid is known to spare vitamin E (tocopherols and tocotrienols) [35], which is another essential nutrient required by infants for healthy development.

Losses of ascorbic acid during the handling and storage of human milk have been studied. In one report, storage at both refrigerator and freezer temperatures led to a significant decrease in the antioxidant capacity of human milk [36]. In related reports, total ascorbic acid levels decreased on average by one-third after 24 hours of storage at 4°C, with wide variations between individuals (6 to 76% and 3 to 100%, respectively with N = 11) [37]. Similarly, in present study ascorbic acid significantly decreased at room temperature and 4^{0}C in day 2 and day 4 respectively but not at -20^{0}c. So, it could be suggested by our research that the antioxidant property of ascorbic acid might be retain when the human milk stored at-20^{0}C. Researchers have investigated the effect of storage on quality and oxidative sensitivity of human milk [38]. Further, storage of human milk and infant formula leads to the formation of lipid peroxidation products, such as lipid peroxides and aldehyde breakdown products [39].

The changes developing during storage are attributable to processes like lipid peroxidation that certainly occur under refrigerating and freezing conditions. Lipid peroxidation is the oxidative degradation of lipids, which leads to free radical formation and impairment of the antioxidant defense system. The malondialdehyde (MDA) concentration, which is a final lipid peroxidation product and therefore a good marker of the degree of the oxidation process, with some important biological effects [40]. Miranda et al [38] reported fresh samples show a lower MDA concentration than the ones kept under both storage conditions: refrigerated and frozen. The study has shown that little oxidation occurs in stored frozen breast milk (-20°C), but oxidation does take place in breast milk stored in a refrigerator (4°C). The present study investigated both forms of storage at 4^0C and -20^0c along with room temperature. The concentration of MDA was significantly increased at room temperature and at4^0C on day 2 and day 4 respectively. Our results revealed that MDA was increased only in refrigerated (4^0C) milk but not in frozen samples (-20^0C). Thus, freezing seems better than refrigeration in order to prevent lipid peroxidation in stored human milk samples.

5. Conclusion

The temperature at which breast milk is stored has little influence on its lactose and protein content. Lipid peroxidation occurred under room temperature and 4°C storage conditions, resulting in decreased in fat content and increased MDA content. As a result, the antioxidant defense weakened, as demonstrated by a decline in ascorbic acid content. Whereas, no notable changes were noticed after four days of storage at -20 degrees Celsius. As a conclusion, the nutritional content of expressed milk is safe while kept at -20 degrees Celsius.

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