

## Original Article



# Macronutrient Analysis of Human Milk according to Storage and Processing in Korean Mother

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### Conflict of Interest

The authors have no financial conflicts of interest.

## ABSTRACT

**Purpose:** As the importance of breastfeeding has been reinforced, human milk is often stored for practical reasons. Therefore, we evaluated optimal storage and processing methods for human milk from a nutritional standpoint.

**Methods:** Human milk samples were collected between June 2017 and February 2018. Also, data about maternal information were collected. Human milk was analyzed for macronutrients and caloric content. The samples were subdivided into groups for nutrient analysis. The control group (fresh milk) was not stored or processed. The other groups (9 groups) consisted of samples analyzed based on different storage temperatures (room temperature, refrigerated, frozen), defrosting methods (bottle warmer, room temperature thawing, microwave oven), and storage period (1 week, 1 month, 2 months) and compared with the control group.

**Results:** There was no statistically significant difference in the nutrient content of human milk among the collected samples. A significant change in the content of macronutrients in milk samples was observed under storage condition at different temperatures for 1 week with subsequent thawing with bottle warmer compared to fresh milk. Under storage at  $-20^{\circ}\text{C}$  for 1 week with subsequent thawing with different defrosting methods, a significant change in the content of macronutrients in milk samples was observed compared to fresh milk. After storage at  $-20^{\circ}\text{C}$  for different periods and thawing with a bottle warmer, a significant change in macronutrient content in milk samples was observed compared to fresh milk regardless of the storage period.

**Conclusion:** Unlike previous guidelines, changes in macronutrient content in milk samples were observed regardless of the method of storing and thawing. Apparently, it is proposed that mothers should feed fresh human milk to their babies without storing.

**Keywords:** Human milk; Breastfeeding; Infant formula; Macronutrients

## INTRODUCTION

Human milk is an important source of nutrition for neonates and infants and plays an important role in growth and development [1,2]. Breastfeeding aids in cognitive development and reduces the incidence of infections, sudden infant death syndrome, obesity, diabetes

mellitus, and leukemia, among others and a reduction in the incidence of maternal postpartum depression has also been hypothesized [3-5]. Despite the development of various formulas, the advantages of human milk cannot be replaced [6]. The World Health Organization recommends exclusive feeding of human milk during the first 6 months of life, based on a systematic review [7].

As more women return to the workforce after childbirth, most of them cannot breastfeed directly. However, unless properly stored and processed, human milk loses its nutrient and immunologic value; accordingly, appropriate guidelines have been proposed for storage and thawing [8-10]. When frozen human milk is thawed, it is recommended to place it in the refrigerator overnight and thaw in the water bath [8]. Defrosting human milk in microwave significantly decreases the activity of immunological factors; hence thawing in a microwave oven is not recommended [8,11]. Instead of a well-equipped human milk bank or hospital, mainly human milk is stored in a home refrigerator or freezer and thawed with a bottle warmer or microwave oven. Previous studies related to changes in macronutrients following storage or treatment of human milk have mostly been conducted at a laboratory level rather than employing actual storage conditions. Most of the reported studies have examined fat or caloric content, and a few studies have evaluated the content of macronutrients, such as carbohydrates, proteins, and lipids [12-15].

Therefore, we analyzed changes in macronutrients that occur when milk is stored and processed under actual conditions in daily life. This is the first Korean study to analyze the nutritional value of human milk according to different household storage and handling conditions.

## MATERIALS AND METHODS

### Human milk and data collection

Samples were collected at our human milk research institute for analysis between June 2017 and February 2018. For accurate caloric measurement, colostrums and human milk samples obtained more than 5 months after delivery were excluded, and samples obtained more than one week after delivery were analyzed.

Maternal information was collected with a questionnaire at the same time as human milk collection. Data collected included age, gestational age at delivery, birth weight, number of births, infant's sex, the method of delivery, and postnatal weight change. Medication history and other clinical information related to human milk were also recorded. Women on high-fat diets, high-protein diets, and nutritional supplements were excluded, as these could affect the nutritional status of milk. Finally, human milk collected from 119 mothers was analyzed.

### Analysis of human milk according to storage and thawing methods

Collected milk was immediately divided into 9 groups, each containing 5–10 mL of human milk. Aliquots were subjected to different storage periods, storage methods, and rewarming methods. The first and second samples were analyzed immediately, but the second sample was subjected to microwave heating for 1 minute. Third, fourth, and fifth samples were stored at different temperatures (room temperature of 10°C–20°C, 2°C, and –20°C, respectively) for 1 week and rewarmed in a bottle warmer (20°C for 3 minutes). In this study, we used “The bottle warmer” (Philips, Amsterdam, the Netherlands) and a water bath machine that is commonly used to defrost human milk or warming artificial milk. Sixth and seventh samples

were stored at  $-20^{\circ}\text{C}$  for 1 week. The sixth sample was rewarmed at room temperature and the seventh sample was rewarmed in a microwave for about 1 minute. Eighth and ninth samples were stored at  $-20^{\circ}\text{C}$  and both were rewarmed in a bottle warmer, but the former was stored for 1 month and the latter for 2 months. The difference in the content of macronutrients between the groups reflected the effects of microwave heating, storage temperature, rewarming methods, and storage period.

### Macronutrient analysis

Analysis of fat, carbohydrate, and protein content was performed with a MIRIS b analyzer (Miris AB, Uppsala, Sweden). MIRIS uses mid-infrared analysis, an indirect physical method that measures the fat, protein, and carbohydrate content through absorption of energy by unique functional groups in the molecules. The mid-infrared analysis is based on the absorbance of radiation by functional chemical groups ( $\text{CH}_2$ ,  $\text{C}=\text{O}$ ,  $\text{CONH}$ ,  $-\text{OH}$ ) that are associated with fat, protein, and carbohydrate. The total nitrogen content in 5–10 mL of milk sample was measured as crude protein, of which total protein was the value exclusive of non-protein nitrogen content. Non-protein nitrogen-containing compounds, including urea, uric acid, creatine, creatinine, amino acids, and nucleotides, comprise up to 25% of human milk nitrogen.

### Statistical analysis

Statistical analysis was performed using SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA). Analysis of variance, Student's *t*-test, Pearson's  $\chi^2$  test, and the paired *t*-test were used to analyze differences between the groups. The level of statistical significance was set at  $p < 0.05$ .

This study was conducted with approval from the Institutional Review Board (IRB) of Chung-Ang University Hospital (IRB No. C2016042).

## RESULTS

### Demographic data

**Table 1** shows demographic data surveyed through questionnaires. The mean age of the 119 mothers was  $31.85 \pm 3.27$  years and the gestational age of infants was  $39.17 \pm 1.15$  weeks. The mean age at the time of delivery was  $22.77 \pm 31.98$  days. The time of maximum human milk extraction was at 06:00 a.m.–12:00 p.m. (42.9%). Eighteen milk specimens were analyzed within 5 minutes of extraction (15.1%), 56 within 5 to 15 minutes of extraction (47.1%), and 45 more than 15 minutes after extraction (37.8%). Birth history and breast side extracted are shown in **Table 1**.

### Initial macronutrient analysis of human milk

Macronutrient analysis of 119 human milk samples revealed the following results:  $68.88 \pm 8.07$  kcal/100 mL, carbohydrate  $7.62 \pm 0.34$  g/100 mL, crude protein  $1.59 \pm 0.36$  g/100 mL, total protein  $1.27 \pm 0.29$  g/100 mL, and fat  $3.44 \pm 0.92$  g/100 mL. Gestational age, birth weight, and method of delivery were not related to the caloric content. There were no statistically significant differences in the caloric content of human milk according to the time of extraction and postnatal weight change.

**Table 1.** General characteristics of the lactating women (n=119)

Variable	Total
Maternal age (yr)	31.85±3.27
Gestational age (wk)	39.17±1.15
Birth weight (kg)	3.24±0.37
Postnatal weight change (kg)	0.48±0.80
Postnatal age (day)	22.77±31.98
The time of human milk extraction	
06:00 a.m.–12:00 p.m.	51 (42.9)
12:00 p.m.–18:00 p.m.	22 (18.5)
18:00 p.m.–24:00 p.m.	22 (18.5)
00:00 p.m.–06:00 a.m.	24 (20.2)
No. of births (1st baby/2nd baby)	100 (84.0)/19 (16.0)
Method of delivery (normal delivery/C-sec)	58 (48.7)/61 (51.3)
The side extracted (left/right/both)	17 (14.3)/15 (12.6)/87 (73.1)
Infant sex (male/female)	64 (53.8)/55 (46.2)

Values are presented as mean±standard deviation or number (%).  
C-sec: Caesarean section.

### Macronutrient changes after microwave heating

Macronutrient analysis of the milk sample after immediate microwave heating for 1 minute after receiving the sample was statistically different from that of fresh human milk (**Table 2**), showing following results: 88.37±18.54 kcal/100 mL, carbohydrate 9.61±1.11 g/100 mL, crude protein 2.38±0.71 g/100 mL, total protein 1.92±0.57 g/100 mL, and fat 4.27±1.60 g/100 mL (all  $p=0.000$ ).

### Macronutrient analysis based on different storage temperature

Human milk was stored for 1 week at different temperatures and subsequently thawed with a bottle warmer. Macronutrient analysis was performed and compared with the results obtained immediately after collection (**Table 3**). When stored at room temperature, human milk analysis showed the following data: 63.63±7.27 kcal/100 mL ( $p=0.000$ ), carbohydrate 7.80±0.44 g/100 mL ( $p=0.000$ ), and fat 2.73±0.88 g/100 mL ( $p=0.000$ ), with statistically significant differences. However, there was no significant difference in protein content. Similar results were observed when sample was stored under refrigeration, as follows: 66.32±7.89 kcal/100 mL ( $p=0.000$ ) and fat 3.11±0.97 g/100 mL ( $p=0.000$ ), with statistically significant differences. However, carbohydrate and protein content were not statistically different. Under frozen storage, results were as follows: 65.47±6.75 kcal/100 mL ( $p=0.000$ ),

**Table 2.** Macronutrient change of human milk after heating for 1 minute with microwave oven (n=119)

Variable	Fresh milk	Microwave oven	$p^*$
Energy (kcal/100 mL)	68.88±8.07	88.37±18.54	0.000
Carbohydrate (g/100 mL)	7.62±0.34	9.61±1.11	0.000
Crude protein (g/100 mL)	1.59±0.36	2.38±0.71	0.000
Total protein (g/100 mL)	1.27±0.29	1.92±0.57	0.000
Fat (g/100 mL)	3.44±0.92	4.27±1.60	0.000

\*Significant findings at  $p<0.05$ .

**Table 3.** Macronutrient analysis according to storage temperature (n=119)

Variable	Fresh milk	Room temperature	$p$	Refrigerated storage	$p$	Frozen storage	$p$
Energy (kcal/100 mL)	68.88±8.07	63.63±7.27	0.000*	66.32±7.89	0.000*	65.47±6.75	0.000*
Carbohydrate (g/100 mL)	7.62±0.34	7.80±0.44	0.000*	7.67±0.39	0.086	7.69±0.32	0.001*
Crude protein (g/100 mL)	1.59±0.36	1.67±0.68	0.173	1.61±0.34	0.406	1.50±0.35	0.001*
Total protein (g/100 mL)	1.27±0.29	1.30±0.31	0.282	1.30±0.28	0.228	1.20±0.29	0.001*
Fat (g/100 mL)	3.44±0.92	2.73±0.88	0.000*	3.11±0.97	0.000*	3.04±0.76	0.000*

\*Significant findings at  $p<0.05$ .

## Macronutrient Analysis of Human Milk

**Table 4.** Macronutrient analysis according to thawing method (n=119)

Variable	Fresh milk	Bottle warmer	<i>p</i>	Room temperature	<i>p</i>	Microwave oven	<i>p</i>
Energy (kcal/100 mL)	68.88±8.07	65.47±6.75	0.000*	65.45±7.45	0.000*	75.29±14.59	0.000*
Carbohydrate (g/100 mL)	7.62±0.34	7.69±0.32	0.001*	7.65±0.34	0.275	8.86±1.00	0.000*
Crude protein (g/100 mL)	1.59±0.36	1.50±0.35	0.001*	1.69±1.46	0.430	2.02±0.80	0.000*
Total protein (g/100 mL)	1.27±0.29	1.20±0.29	0.001*	1.25±0.29	0.397	1.59±0.46	0.000*
Fat (g/100 mL)	3.44±0.92	3.04±0.76	0.000*	3.02±0.82	0.000*	3.37±1.23	0.428

\*Significant findings at  $p < 0.05$ .

**Table 5.** Macronutrient analysis according to storage period (n=104)

Variable	Fresh milk	1 wk	<i>p</i>	1 mo	<i>p</i>	2 mo	<i>p</i>
Energy (kcal/100 mL)	69.02±7.91	65.80±6.64	0.000*	64.94±6.69	0.000*	66.15±6.96	0.000*
Carbohydrate (g/100 mL)	7.63±0.36	7.70±0.33	0.001*	7.76±0.25	0.000*	7.78±0.29	0.000*
Crude protein (g/100 mL)	1.59±0.37	1.52±0.36	0.007*	1.50±0.34	0.003*	1.57±0.34	0.502
Total protein (g/100 mL)	1.27±0.30	1.21±0.29	0.004*	1.20±0.28	0.002*	1.26±0.28	0.545
Fat (g/100 mL)	3.45±0.91	3.07±0.75	0.000*	2.95±0.74	0.000*	3.05±0.75	0.000*

\*Significant findings at  $p < 0.05$ .

carbohydrate 7.69±0.32 g/100 mL ( $p=0.001$ ), crude protein 1.50±0.35 g/100 mL ( $p=0.001$ ), total protein 1.20±0.29 g/100 mL ( $p=0.001$ ), and fat 3.04±0.76 g/100 mL ( $p=0.000$ ), with statistically significant differences.

### Analysis of the macronutrients according to the thawing method

Human milk was frozen at  $-20^{\circ}\text{C}$  for 1 week and analyzed for nutritional content according to the thawing method (Table 4). In group 5, change in the content of macronutrients was observed after thawing with a bottle warmer. However, thawing at room temperature revealed the following results: 65.45±7.45 kcal/100 mL ( $p=0.000$ ) and fat 3.02±0.82 g/100 mL ( $p=0.000$ ) with statistically significant differences. There were no significant differences in carbohydrate and protein content.

Rapid microwave thawing revealed the following results: 75.29±14.59 kcal/100 mL ( $p=0.000$ ), carbohydrate 8.86±1.00 g/100 mL ( $p=0.000$ ), crude protein 2.02±0.80 g/100 mL ( $p=0.000$ ), total protein 1.59±0.46 g/100 mL ( $p=0.000$ ), and fat 3.37±1.23 g/100 mL ( $p=0.428$ ), with statistically significant differences. There was no significant difference in fat content.

### Analysis of the macronutrients according to the storage period

Human milk was stored frozen at  $-20^{\circ}\text{C}$  for 1 week, 1 month, or 2 months, and analyzed for nutritional content after thawing with a bottle warmer (Table 5). Among 119 samples, 14 were lost during storage and 105 were analyzed. After storage for 1 or 4 weeks, change in nutrient content was observed compared to fresh human milk. Storage for 8 weeks resulted in a significant change in the content of all the nutrients except for protein content compared to fresh human milk.

## DISCUSSION

Human milk is composed of various micronutrients, bioactive compounds, growth factors, and immunologic factors, as well as macronutrients such as carbohydrates, proteins, and fats [16]. The optimal method for providing these components to infants is direct breastfeeding without storage. However, most of the times, direct feeding may not be possible. Apparently, various guidelines for the storage of human milk have been proposed. Typically, milk can be

stored for 3–4 hours at room temperature (16°C–19°C), 72 hours in a refrigerator (4°C), 5–8 days in a refrigerator under very clean conditions, and up to 12 months in a freezer (-17°C) [8-10].

Holder pasteurization is recommended to maintain human milk in a clean and nutritionally optimal state in a human milk bank [12,17-20]. However, as noted, it is difficult to apply these guidelines under home conditions. Therefore, we examined the effect of home storage and thawing methods on the nutritional value of human milk.

In our study, an increase in the content of macronutrients may not be indicative of actual nutrient value but may have reflected structural changes in nutrients that were increased by heating. When human milk is heated rapidly, nutrients are denatured or polymerized, resulting in structural change with an apparent increase in the content [21-23]. Therefore, while interpreting the results of the subsequent analysis, we considered the change itself rather than the increase or decrease in the results. Furthermore, the results obtained under different storage methods were similar. Total caloric content was significantly changed in all the groups compared to fresh milk. Nutrient changes were evident even for the samples that were subjected to different storage temperature, period, and defrosting methods as recommended in the guidelines, compared to fresh milk.

Bertino et al. [13] investigated the effect of prolonged refrigeration of human milk on lipid content and found no change in the fatty acid composition after up to 96 hours. In our study, the fat content was significantly decreased in samples that were stored for 1 week in a refrigerator. It may be difficult to identify the differences in the nutrient content according to the storage environment and thawing method, but based on the results, maximum appropriate refrigerated shelf life is hypothesized to be 4 days. This requires further study.

Thatrimontrichai et al. [14] measured the fat content of fresh human milk, milk stored at -20°C for 30 days, milk subjected to refrigerated thawing, and water bath thawing. Both samples showed a significant difference in fat content compared to fresh milk and less fat loss was observed subsequent to refrigeration for 24 hours. Our study did not examine the effect of refrigerated thawing, which requires additional research in the future.

Nevertheless, there were some limitations in our study. A greater number of specimens would have yielded more reliable results. More detailed comparisons would have been possible if the classification of storage status had been more detailed, with the inclusion of more thawing methods. We could not determine the mechanism by which different storage conditions led to changes in the content of the macronutrients. In addition, since only macronutrients were examined, changes in micronutrients, microbiomes, and other human milk components remain as unknown and require further study.

Nonetheless, this is the first study to analyze the changes in the content of the macronutrients in human milk according to various household storage and thawing methods in Korea. Presence of a breastfeeding room or milking room in the workplace is gradually being reinforced and is referred to as a mother-friendly workplace. Based on these policies, the term “family-friendly corporation” has emerged. Many mothers store the expressed milk in the milking room refrigerator, bring it home, freeze it, or feed it immediately. They believe that there will be little change in nutrients due to refrigeration and freezing storage. According to the present study, optimal delivery of macronutrients is achieved with the use of fresh human milk, since even storage and thawing as per the guidelines result in nutritional

composition changes compared to fresh human milk. In conclusion, we recommend that mothers should feed fresh human milk to their babies without storing. In situations where they need to store their milk inevitably, following the previous guidelines will be the best way to minimize the changes in the nutrient composition. In 2017, Kim [24] reviewed the important role of training and education about human milk feeding. In addition, education on storage of human milk is very important.

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