

## **EFFECT OF TEMPERATURE AND STORAGE DURATION ON LACTOSE, PROTEIN AND FAT CONTENT OF BREAST MILK**

**Muhammad Iqbal<sup>1,\*</sup>, Lily A. Lestari<sup>2</sup>, Weny Kurdanti<sup>3</sup>, Nur Lathifah Mardiyati<sup>4</sup>**

<sup>1</sup> Department of Nutrition Science, Faculty of Health Science, University of Darussalam  
Gontor, Ponorogo, Indonesia

<sup>2</sup> Department of Health Nutrition, Faculty of Medicine, Universitas Gadjah Mada,  
Yogyakarta, Indonesia

<sup>3</sup>Health Polytechnic of Health Ministry Yogyakarta, Indonesia

<sup>4</sup> Department of Nutrition Science, Faculty of Health Science, Universitas Muhammadiyah  
Surakarta

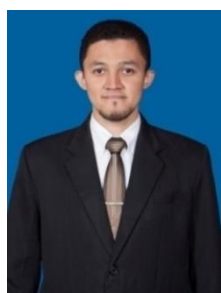
*iqbalbasagili@gmail.com (Muhammad Iqbal)*

### **Abstract**

Many working mothers stop breastfeeding their babies while actually breast milk can be stored and fed to babies while mothers are working, as long as the storing process is carried out properly. Thus, it is important to understand the most appropriate way to store breast milk. The objective of the study was to assess the influence of different temperatures and storing durations on the contents of lactose, protein and fat in breast milk. This research was an experimental study conducted in the laboratory with a complete randomized design. Mature breast milk samples were obtained from 21 volunteer mothers, at least two weeks after they delivered their babies. Samples were divided into three treatment-groups of storing temperatures: room, refrigerator and freezer temperatures. The measurement of the contents of lactose, protein and fat performed in each sample on the 2<sup>nd</sup> and 3<sup>rd</sup> day. Statistical analysis was carried out by Anova with the degree of significance of 99% and followed with Duncan tests. There were insignificant differences of storage temperatures ( $P>0.01$ ) in the lactose, protein and fat contents of breast milk, with p-value of 0.893, 0.973, 0.950, respectively. Furthermore, there was insignificant impact of the storing duration on lactose content of breast milk with p-value of 0.096. On the contrary, the storing duration affected the protein and fat contents of breast milk significantly, each showed p-value: 0.001. The temperature of storage did not affect the contents of lactose, protein and fat of breast milk. Meanwhile, the storage duration influenced the protein and fat content. Based on this research, breast milk can be stored up to 72 hours in the refrigerator or freezer temperature to keep the nutrient contents intact.

**Keywords: breast milk, storing, nutrient quality, temperature, duration.**

### **Presenting Author's biography**



Muhammad Iqbal is a junior lecturer in Nutrition Department, The Darussalam University of Gontor since 2015. His researches focus on Food Service and Breast Milk. Iqbal received his Bachelor of Nutrition and his Master of Public Health from Universitas Gadjah Mada. He is also one of the journal reviewers in Indonesian Nutrition Student Journal.

## **BACKGROUND**

Breast Milk is a food designed to fulfill the energy requirement of the babies. It contains both specific and non-specific immune factors that help newborn infants to consolidate their immature immune systems, in addition to protect their body from infection as well [1]. Bioactive proteins in breast milk are likely to contribute to the advantages of breast-feeding through enzyme activities, enhancement of nutrient absorption, growth stimulation, modulation of the immune system and defence system against pathogens [2]. Breast milk also contains more than 200 fundamental elements that are proportional and balanced one another [3].

The main components of breast milk are water, protein, carbohydrates and fats. In addition, breast milk contains an inorganic substance in the form of mineral and organic substances such as vitamins, acids, antioxidants, pigments and flavor components. Protein content in food determines the quality of the food. The protein content of the milk can also be used as a standard assessment of the nutritional quality of breast milk [5].

In assessing the nutritional quality, the macro-nutrients should be the primary priority before the examination on the micro-nutrients. All kinds of macro-nutrients are contained in breast milk, including carbohydrates, proteins and fats. The main type of carbohydrate found in breast milk is lactose [4]. Breast milk contains lactose as much as 7-7.5% [5] and its content depends on the mother [6] and breastfeeding state [7].

The main protein in breast milk is whey, whereas the main protein in cow's milk is casein. Whey and casein ratio of breast milk is 60:40 while the ratio in cow's milk is 20:80. It surely makes breast milk is more advantageous as it will be easier for the baby to digest whey than casein [4]. In addition, it is also one of the reasons why breast milk is referred to as a protein that is digestible [2]. Another protein contained in breast milk is an alpha-lactalbumin, while cow's milk contains lactoglobulin and bovine serum albumin which frequently cause allergies on the babies [3].

Nowadays, a large number of working mothers continue to breastfeed for so many reasons [12]. In Indonesia, the number of female labor force showed a rising trend with a rapid increase compared to the number of male labor force. It is attributed to a significant increase of the number of females in addition to the vast requirement on employment and the higher level of women's education. Based on the population census of 2010, the number of female labor force was 84.7 million people of the total population of 118 million women [13]. From the fact, the participation rate of women in the work force was 51.69% and it was less than the number of men's involvement in the work force of 84.17%. In the development context, women are also considered to have the same responsibilities with men, especially the role in the national development [14].

Factors that become the obstacles to breastfeed for working mothers is never be an excuse for them to stop breastfeeding. It is due to the adequate knowledge related to breastfeeding, available fixtures in storing breast milk, and support from the work environment. As a result, working mothers are able to exclusively breastfeed their babies as breastfeeding itself is actually a natural process that should be done by every mother to her baby [3].

Basically, there is a solution for women who have problems with breastfeeding because they have to work outside the home and cannot perform it directly. Working mother can store breast milk which can be given to the baby when the mother is unavailable at home [3]. In addition, the storage is also required as mother plans to delay the feeding [14]. Breast milk storage is an innovation which should be developed and further investigated in order to find

the most appropriate method to store the breast milk to minimize the loss of nutrients contained in breast milk. Subsequently, it is expected that in the future, it will be a solution for mothers who cannot breastfeed their babies directly.

Stored breast milk is frequently less than optimal storage conditions [8]. It is due to the proteolysis reactions can also undergo as the denaturation of proteins. Denaturation is the loss of properties of higher structures by hydrogen bonds degradation and other secondary forces that condemn the molecule. Proteins can be denatured if there are changes in temperature [9].

Optimal storage conditions are required as breast milk is a product of human which in this case is classified as a mammal. Vegetable food ingredients are relatively more durable than animal shelf time. It means the milk as the animal products has relatively short time for storage [5].

Storage process can preserve the milk for some time. One of the goals of food preservation is to maintain the quality of foodstuffs. The quality of food itself can be examined from its nutritional quality, as shown by the protein content in food so that the protein can determine the quality of foodstuffs [5]. Protein is just one indicator of the food quality as there are many other indicators which should be considered in assessing the nutritional quality of food, especially in breast milk. The main components of macro-nutrient in breast milk are lactose, protein and fat. Breast milk has higher quantities of those components as its nutritional content in comparable to other products [15]. It is necessary to recognize the extent of the stability of the macro-nutrients (lactose, protein and fat) of breast milk during storage process.

Furthermore, the nutritional quality of breast milk should be noticed in association with the influence of a wide variety methods and duration of the storage. It is presumed that not all conditions and durations of storage provide the guarantee of similar nutritional quality of breast milk. Conditions and durations of storage have different values of nutritional quality. Hence, it is necessary to investigate the conditions and durations of breast milk storage which are capable in maintaining the stability of the nutrients quality, it might address the issues regarding with the storage of breast milk. However, the maintenance of the nutrients quality of breast milk is important to keep the nutrients intact before it is given to the babies.

## **METHODS**

### **Ethical clearance**

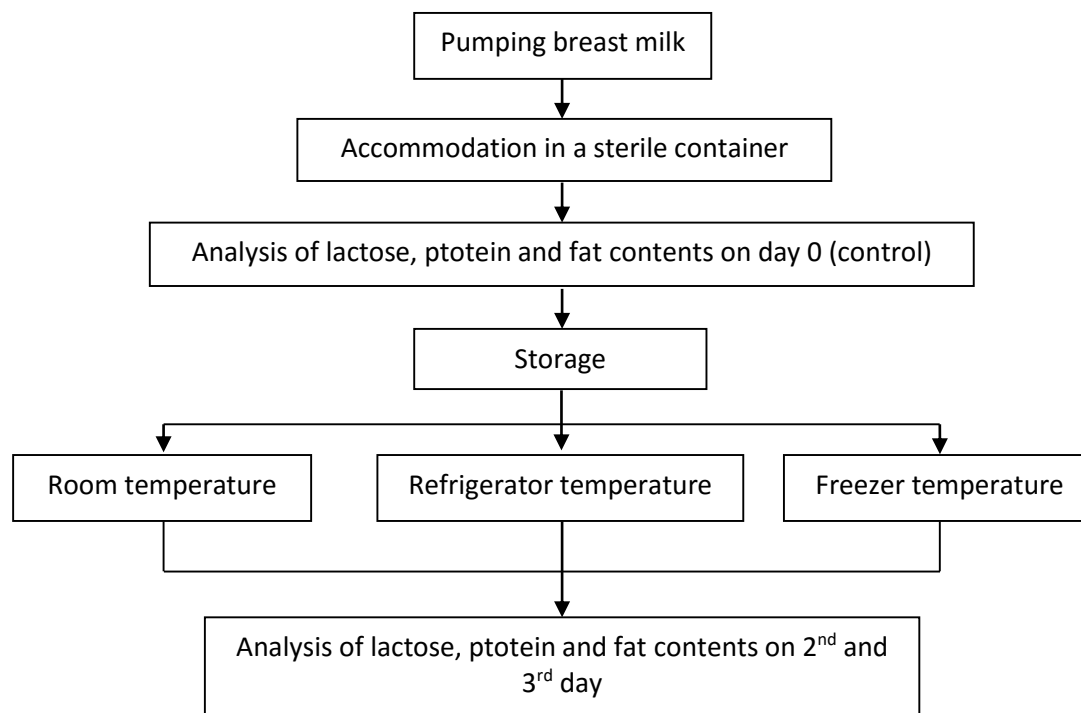
This study was an experimental research performed at the laboratory with the design of completely randomized design (CRD). It has been approved by the Medical and Health Research Ethics Committee (MHREC), Universitas Gadjah Mada and all volunteers were provided by written informed consent before participating in this study.

### **Breast Milk Collection**

Breast milks obtained from the volunteers were predefined and collected in sterile containers. Breast milks were collected to measure the lactose, protein and fat contents to determine the nutrient levels prior to the analysis. The participants in this study were mother who delivered their babies at least 2 weeks before the samples' collection. The consideration was the immature and transition breast milk has unstable nutritional contents.

Each participant was equipped with sterile breast pump to collect the breast milk which was further homogenized. Subsequently, the samples of breast milk were divided into

three treatment groups: 1) Group A: Storage at room temperature; 2) Group B: Storage at refrigerator temperature (4<sup>0</sup>C); 3) Group C: Storage at freezer temperature.



**Picture 1.** The Scheme of Sample Analysis.

### **Analysis of NutrientContents**

Breast milk samples were analyzed 2 times for each treatment on day 2 and 3. The analysis mechanism was the samples were stored in accordance to the treatment and then the lactose, protein and fat contents of the samples were analyzed. Subsequently, the rest of the samples were stored based on the treatment. In the freezer storage, the containers were placed separately to avoid repetitive thawing. The lactose content was analyzed by the method that has been done by Sudarmadji *et al.* (1984), titration filtrate screening results from the content-free milk in addition to lactose [16], the protein content was analyzed by the Kjeldahl method, while the fat content was analyzed by Babcock method [11].

### **Statistical analysis**

Data presented in the table provides the relationship analysis between the storage type levels and the lactose, protein and fat contents after the breast milk was stored at the 2<sup>nd</sup> and 3<sup>rd</sup> day. The levels of observed nutrients (lactose/protein/fat) in the three different variations of storage were statistically examined using analysis of variance (ANOVA) with Confidence Interval (CI) of 99%. It was followed by Duncan's new multiple range test (DMRT) to test the pairwise comparisons between several means [17].

### **Results**

The study was commenced by collecting the samples of breast milk from 21 participants (n = 21) aged between 22 to 39 year-old in Wirobrajan District. The study took place approximately 10 days. The lactose content of samples stored in the room, refrigerator, and freezer temperature was evidenced to decline in the 3<sup>rd</sup> day. Descriptively, the samples'

lactose contents in refrigerator and freezer storage increased in comparable to the control on the 3<sup>rd</sup> day, but it did not affect the significance of the research results.

**Table 1.** The level of lactose, protein and fat contents in a variety of methods and storage durations.

Nutrient contents	Temperature	Storage Duration		
		Day 0	Day 2	Day 3
Lactose Level $\pm$ SD (g/100mL)	Room	17.50 $\pm$ 0	15 $\pm$ 1,69	12,60 $\pm$ 0,99
	Refrigerator	17.50 $\pm$ 0	13.80 $\pm$ 0	15.35 $\pm$ 4.60
	Freezer	17.50 $\pm$ 0	12,3 $\pm$ 7.50	18.00 $\pm$ 7.50
Protein Level $\pm$ SD (g/100mL)	Room	3.40 $\pm$ 0	1.8 $\pm$ 0.14	1.90 $\pm$ 0
	Refrigerator	3.40 $\pm$ 0	1.95 $\pm$ 0.07	1.95 $\pm$ 0.07
	Freezer	3.40 $\pm$ 0	1.90 $\pm$ 0	2.10 $\pm$ 0.28
Fat Level $\pm$ SD (g/100mL)	Room	2.5 $\pm$ 0	2.5 $\pm$ 0	2.00 $\pm$ 0
	Refrigerator	2.5 $\pm$ 0	2.5 $\pm$ 0	1.75 $\pm$ 0.35
	Freezer	2.5 $\pm$ 0	3.00 $\pm$ 0.70	1.5 $\pm$ 0

Statistically, there were insignificant differences obtained from the storage temperatures ( $P > 0,01$ ) on the lactose, protein and fat contents of breast milk, with p-value of 0.893, 0.973, 0.950, respectively. Furthermore, there was insignificant impact of the storing duration on the lactose content of breast milk with p-value of 0.096. On the contrary, the duration of breast milk storage affected the protein and fat contents of breast milk, significantly, which was expressed by p-value = 0.001.

## DISCUSSION

This study indicated that there is no effect between the storage temperature and the level of lactose, protein and fat content of breast milk. The duration of storage also insignificantly affects the lactose content of breast milk but has an effect on the protein and fat content of breast milk. The presence of simple sugars such as glucose on breast milk has an effect on the breast milk quality as glucose is the main dietary source of microbes, particularly bacteria. Bacterial cells tend to use a carbon source with the simplest molecular structure such as glucose available in the cells. Therefore, if the bacterial cells are cultured in medium containing two kinds of different carbon source structures, such as glucose (monosaccharide) and lactose (disaccharide), then the bacterial cells will use glucose first because the molecular structure is simpler compared to lactose [18, 19].

The variation of storage temperature have insignificant effect on the protein content of the milk. According Makfoeld *et al.* 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 [20]. 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 4°C for 48

hours [10]. The storage of breast milk in the freezer does not have a major influence on the protein level, so that the milk can be stored safely for 72 hours at a temperature of 4-6<sup>0</sup>C [21]. The results of this study support the previous study stated that the temperature on different types of storage did not affect the levels of protein.

Breast milk contains the enzyme lipase which serves to digest fats [3, 22]. The process that occurs with the help of the enzyme lipase is breakdown of triglycerides into diglycerides and later became monoglycerides [4, 22]. Theoretically, the levels of fat content in complex form was diminished but due to the analysis of the levels of fat content was carried out using Babcock in this study, the results indicated the quantity of total fat instead of specifically on a particular type of fat with a consideration that the fat had been digested was also counted [3]. Storage duration significantly influenced the changes in the fat content of breast milk, it was suspected because of the activity of lipolytic bacteria which produce the lipase enzyme in breast milk. Lipase breaks down the fat into fatty acids and glycerol [23]. Another study also explained that lipolysis runs very fast starting from the first hour of storage and process reached 8% at 24 hours of storage [8]. It is required to investigate further on the effect of storage duration on the fat content of breast milk.

In conclusion, the storage temperature has no effect on the level of lactose, protein and fat contents of breast milk. The duration of storage do not affect the lactose content of breast milk but affects the protein and fat content of breast milk. Based on this research, breast milk is evidenced to have its beneficial nutrients even after it is stored at refrigerator and freezer temperatures for 72 hours.

## REFERENCES

- [1] Mahan, L. K. and Sylvia, E. S. (2004). *Krause's: Food, Nutrition, & Diet Therapy 11th edition*. Pennsylvania: Saunders.
- [2] Lönnerdal, Bo. (2013) Bioactive Proteins in Breast Milk. *Journal of Paediatrics and Child Health*: 49 (Suppl. 1), 1–7.
- [3] Roesli, Utami. (2000). *Mengenal ASI Eksklusif*. Jakarta: Trubus Agriwidya.
- [4] Soetjiningsih. (1997). *ASI Petunjuk untuk Tenaga Kesehatan*. Jakarta: Penerbit Buku Kedokteran EGC.
- [5] Widyani, R. and Tety Suciati. (2008). *Prinsip Pengawetan Pangan Ed. Tahun 2008*. Cirebon: Penerbit Swagati Press.
- [6] McSweeney, Paul. 2009. *Advanced Dairy Chemistry: Volume 3: Lactose, Water, Salts and Minor Constituents*. New York: Springer science-bussiness media.
- [7] Kacew, S., Lambert, G. H. (1997). *Environmental Toxicology and Pharmacology of Human Development*. USA: Taylor & Franchis.
- [8] Hamosh, M., Lorie, A.E., Darren, R.P., Theresa, R.H. and Paul, H. (1996). Breastfeeding and the Working Mother: Effect of Time and Temperature of Short-term Storage on Proteolysis, and Bacterial Growth in Milk. *Pediatrics*, 97, pp. 492-498.
- [9] Fessenden, R.J. and Fessenden, J.S. (1982). *Kimia Organik. 3rd ed. Pudjaatmaka, A.H. ed*. Jakarta: Erlangga.

- [10] Ogundele, M. O. (2000). Techniques for the Storage of Human Breast Milk: Implication for Anti-microbial Function and Safety of Stored Milk. *Eur J Pediatr*, 159, pp. 759-797.
- [11] Herreid, E. O. (1942). The Babcock Test: A Review of the Literature. *Jour. Dairy Sci.*: 25 335-370.
- [12] Tim PP-ASI RSUP DR. Sardjito/ FK UGM. (1994). *Semiloka Manajemen Laktasi Puskesmas-TT DIY*. Yogyakarta: RSUP DR. Sardjito
- [13] Statistics Indonesia. (2010). *The Number of Labour Force Achieve 107. 7 Million People*. Available at: <http://sp2010.bps.go.id>.
- [14] Anonymous. (2009). *Analisa Faktor-Faktor yang Mempengaruhi Curahan Waktu Kerja Wanita (Studi Kasus pada Sentra Industri Kecil di Desa Sukodono Kecamatan Gresik Kabupaten Gresik)*. Available at <http://jurnalskripsi.com/analisa-faktorfaktor-yang-mempengaruhi-curahan-waktu-kerja-wanita-studi-kasus-padasentra-industri-kecil-di-desa-sukodono-kecamatan-gresik-kabupaten-gresikpdf.htm>
- [15] Nestlé. (2007). *Agenda 2007 Nestlé Nutrition : Breastfeeding Estimates of the Concentrations of Nutrients in Mature Human Milk*. Jakarta: PT. Nestlé Indonesia.
- [16] Sudarmadji, Slamet., Bambang H., Suhardi. (1997). *Prosedur Analisa untuk Bahan Makanan dan Pertanian, edisi keempat*. Yogyakarta: Liberty.
- [17] Santosa, P. B., Ashari. (2005). *Analisis Statistik dengan Microsoft Excel & SPSS*. Penerbit Andi: Yogyakarta
- [18] Yuwono, Triwibowo. (2006). *Biologi Molekuler*. Jakarta: Erlangga.
- [19] Cavalli C., Cecilia T., Frederick C. Battaglia, Giulio B. (2006). Free Sugar and Sugar Alcohol Concentrations in Human Breast Milk. *Journal of Pediatric Gastroenterology and Nutrition*, 42, pp 215–221.