Universitas Katolik Widya Mandala Surabaya Journal, Available at: https://journal.ukwms.ac.id/index.php/index

Jurnal Widya Medika

Journal Home Page: https://journal.ukwms.ac.id/index.php/JWM
Print ISSN: 2338-0373; Online ISSN: 2623-2723

Original Research Article

COMPARISON OF MICROPLASTIC PARTICLES IN BREAST MILK STORED WITH BREASTMILK STORAGE BAGS AT DIFFERENT TEMPERATURES

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DOI:
https://doi.org/10.33508/jwm.v11i1.7496

Article History:
Received, May 16th, 2025
Revised, June 25th, 2025
Accepted, June 23rd, 2025
Available Online: July 1st, 2025

ABSTRACT

MPs are plastic particles less than 5 mm in size. MPs can enter through the mouth and into the digestive tract, circulate throughout the body's tissues including breast tissue. Research on the size of particles that can be deposited in adipose tissue has not yet been conducted. This quasi-experimental research aims to explain differences in levels of microplastic particles due to differences in processing temperatures in breast milk in storage bags. Breast milk samples were taken from breastfeeding mothers at the Mulyorejo Community Health Center, Surabaya. The breast milk was put into plastic bags for the study group and glass tubes for the control group. Samples will be separated based on breast milk storage temperature and as a control group. The sample will be extracted and examined. After that, additional inspection tests were carried out in the form of micro-FTIR. It was found that there was no significant difference in MPs particle levels with respect to differences in breast milk processing temperatures in storage bags. However, there were significant differences between the control group and the study group.

Keywords: *Breast milk, Microplastics, Temperature*

INTRODUCTION

Microplastics (MPs) are plastic particles less than 5 mm.[1] MPs can enter the body in several ways, namely ingestion, inhalation, or direct contact through the skin with MP particles.[2] Furthermore, MPs particles can circulate to all body tissues, such as muscles, liver, and brain[3], including breast tissue (mammary tissue). Until now there have been no studies examining the size of particles that can be deposited in adipose tissue, but some studies show that solid particles with micro-diameters can penetrate various tissues of the human body.[4]

MPs particles can enter through the blood-milk barrier (BMB) through active transport.[5] The presence of solid particles such as MPs particles in breast tissue can be transmitted from mother to baby through direct transmission during breastfeeding.[6] In

addition, transmission of MPs particles in infants can also occur due to storage of breast milk (hereinafter abbreviated as breast milk) using plastic materials due to direct contamination of breast milk by MPs particles.[7]

Breast milk plays an important role in the growth and development of babies as the main source of nutrition in early life.[8] According to the World Health Organization (WHO), exclusive breastfeeding is given for at least 6 months to 2 years or so after giving birth.[8] Breastfeeding can be done indirectly; namely, breast milk is accommodated in containers, which are then frozen. When it will be used, breast milk will be thawed by leaving it at room temperature or heated in water with the bag.[8] It is known that plastics heated at >70ºC can cause fragmentation of MPs particles.[9] The higher the processing temperature, the more fragmentation particles it can produce.[10]

Periyasami's research (2022) writes that the higher the temperature, the release of fragments (1 μm - 5 mm) will increase.[10] Although not yet statistically significant between temperatures of 25ºC, 40ºC, 60ºC, and 80ºC.[10] It is recommended to use low temperatures to reduce the release of plastic fragmentation during the process.[10] Referring to this advice, it is necessary to know the safe temperature for processing breast milk before giving it to infants, because the presence of MP contamination in breast milk has the potential to cause health impacts on babies due to toxic materials in MPs.[11] Until now, research on MP levels in frozen breast milk -4ºC, thawed at a chiller temperature of 4ºC, and immersed in water at 40ºC has never been conducted, as well as research on the impact of exposure to MP particles on children's health.

MATERIAL AND METHODS

This study employed a quasi-experimental design. The population consisted of breastfeeding mothers who visited the Maternal and Child Health Clinic at Mulyorejo Health Center (Puskesmas Mulyorejo), Surabaya. The technique of measuring MPs in breast milk is by taking breast milk samples on the same day of the experiment. Breast milk samples were taken, as much as 20 cc, and divided into four different places. Fresh breast milk samples were put into test tubes, and a control group was examined immediately after sampling. Breast milk samples in the study group will be put into breast milk bags and stored in the freezer (-4°C) first before examination. Five cc of breast milk is stored in a breast milk bag and allowed to remain in the freezer.[4,12] Five cc of breast milk is put into a breast milk bag and placed into a chiller.

Then, the remaining five cc are put into a breast milk bag and heated in a basin of water with a temperature of 40°C. The duration of breast milk storage is at least one day. Extraction begins with tissue destruction with chemicals in the form of a solution of 10% potassium hydroxide (KOH) and 67% HNO_3 of 1 cc each and added to the prepared breast milk sample.[12] After that, let it stand for 2x24 hours, and the sample is then separated with a centrifuge at 2300 rpm for 2 minutes.[12] After that, the supernatant liquid is filtered through the filter membrane (millipore 0.45 μm).[12] Then, the filter paper is placed in a closed petri dish to prevent contamination and dried at 40°C overnight.[12] Preparat brought in dry conditions.[13]

Next, sample detection was carried out with a Nikon Eclipse E100 binocular microscope of 10-micron magnification to measure levels and diameters and determine the shape of particles.[12] The calculation of levels is carried out on 5 fields of view (1 gridded is 1 fields of view).[12] One sample will be subjected to further examination by micro-Fourier Transform Infrared Spectroscopy to confirm the presence of MPs.

Furthermore, the data entry process in the form of numbers is entered into the Statistical Package for the Social Sciences program version 26. Data analysis to test data normality using Shapiro-Wilk. The data were not normally distributed, so we used the Kruskal-Wallis comparative test. Both statistical tests are considered meaningful if a p-value of <0.05 is obtained.

RESULTS AND DISCUSSION

The results of MPs levels on temperature differences were analyzed by researchers using the computer statistical program. Calculation of mean and standard deviation is carried out as a descriptive analysis of the sample. Furthermore, data analysis is carried out to test the normality of the data using the Shapiro-Wilk test, and if it is known to be abnormal, the comparative test will use the Kruskal-Wallis test. The unit used is particles/5 cc of breast milk.

Table 1. Descriptive Analysis of MPs Level

Group	Mean ± standard deviation of MPs (particle/5 cc breast milk
Control (Fresh breast milk)	1,41 ± 1,50
Breast milk in bags and stored in the freezer -4°C	8,07 ± 6,36
Frozen breast milk that was thawed at 4°C	9,83 ± 10,12
Frozen breast milk that was thawed at 40°C	6,07 ± 5,46

The results of descriptive analysis in 4 groups of breast milk samples showed that the mean and standard deviation of MPs levels were the highest in the temperature group of 4°C, with levels of 9.83 ± 10.12 particles/5 cc, and the lowest in the control group, with levels of 1.41 ± 1.50 particles/5 cc. All groups put into breast milk bags had higher MP results than breast milk in the control group placed in glass tubes. The results showed that the average temperature group of -4°C and 4°C was similar, which was between 8.07 and 9.83 particles/5 cc of breast milk. Levels were found to be slightly lower in the 40°C group of 6.07 MPs/5 cc particles of breast milk.

Diameter measurements on filter paper were carried out by researchers using a Nikon Eclipse Ci-L-DS-F12-L3 binocular microscope of each sample at 5 LP. Diameter measurements are carried out using 2 different types of magnification, namely 40X and200X. The minimum diameter measured at 200X magnification and the maximum diameter is measured at 40X magnification. Diameter is measured in mm. The results of measuring the diameter on MPs particles obtained results of at least 0.01 mm and a maximum of 0.68 mm. The smallest form of MPs particles is fragments, and the largest form of MPs particles is filaments.

Table 2. Descriptive Analysis of MPs Shape

Group	Mean ± standard deviation of MPs Shape	
	Fragment	Filament
Control (Fresh breast milk)	1,28 ± 1,41	0,15 ± 0,42
Breast milk in bags and stored in the freezer -4°C	7,22 ± 6,01	0,83 ± 1,08
Frozen breast milk that was thawed at 4°C	8,43 ± 9,06	1,39 ± 1,60
Frozen breast milk that was thawed at 40°C	4,50 ± 4,86	1,52 ± 1,57

The results of descriptive analysis of particle shapes showed that MPs were only found in fragment and filament forms. In all groups, fragment forms are found more than filaments. In accordance with the results of the MPs description, the mean and standard deviation of the most MPs forms were found, namely fragments in the temperature group of 4°C.

The results of the normality test using the Shapiro-Wilk method showed that the levels of MPs in this study were not normally distributed, as the P-value was less than 0.05 (P=0,001). Therefore, the Kruskal-Wallis test was used for further analysis. The detailed results are presented in the following table 3.

Tabel 3. Kruskal Wallis Test

Group	Statistical Analysis (P value)			
	G0	G1	G2	G3
G0	-	0,001	0,001	0,001
G1	0,001	-	0,931	0,074
G2	0,001	0,931	-	0,177
G3	0,001	0,074	0,177	-

Table Caption: Group 0: Fresh breast milk (control); Group 1: Breast milk stored in bags and frozen at -4°C; Group 2: Frozen breast milk thawed at 4°C; Group 3: Frozen breast milk thawed at 40°C.

The normality test using Shapiro-Wilk showed that the data were not normally distributed, so the comparative test using Kruskal-Wallis in the control group and the study group -4°C, 4°C, and 40°C obtained P = 0.000 which means there is a significant difference between the MPs levels of the control group and the study group. This is because one of them is because the control group uses glass materials while the study group uses plastic materials. In the comparison of the study group between temperatures of -4°C with temperatures of 4°C and 40°C obtained results of 0.931 and 0.074 were obtained, which means there is no significant difference between study groups. In addition, the ratio at 4°C and 40°C shows a result of 0.177, which means there is no significant difference in MPs levels between 4°C and 40°C. The results of statistical analysis show that there is a significant difference in MPs levels against temperature differences. Meanwhile, in the study group with temperatures of -4° C, 4° C, and 40° C there was no significant difference in MPs levels against temperature differences.

The results of micro-FTIR examination found the highest levels of MP polymer types, namely Nylon with levels of 56.67% and the least, namely Aramid, Polymer Additive (DLTDP), Ethylene Vinyl Acetate, Polymer Additive (Irganox 1076), and Poly (Trimethyl Hexamethylene Terephthalamide) with levels of 0.42%.

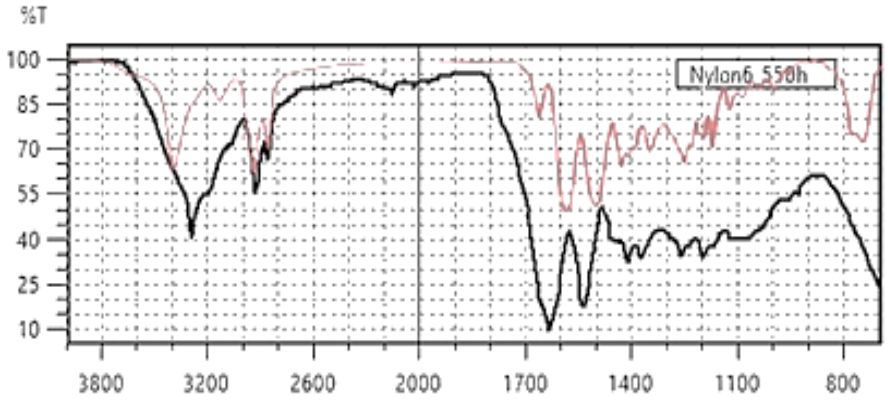

Figure 1. Normal Wave Graph and Nylon Micro-FTIR Result

Similar results were found in Jayadi et al. (2023), discussing the content of PP-type MPs in plastic milk bottles. Samples are taken, as much as 1000 cc in each sample, shaken, and then processed. The results showed that the 6 samples analyzed did not contain PP-type MPs. This is

because PP has a melting point of 130°C-171°C, so at the temperature of this study, it still has not reached the melting point and has not fully decomposed. Munno (2018) said that it is known that plastics heated at a temperature of >70ºC can cause fragmentation of MPs particles.[9]

The higher the processing temperature, the more fragmentation particles it can produce.[10] This shows that the temperature used in this study has still not reached the point of microplastic release. Periyasami's research (2022) writes that the higher the temperature, the more the release of fragments (1 µm-5 mm) will increase.[11] Although not yet statistically significant between temperatures of 25ºC, 40ºC, 60ºC, and 80ºC.[11] It is recommended to use low temperatures to reduce the release of plastic fragmentation during the process.[11]

Different results were obtained in the study of Munno et al. (2017), which discusses the impact of temperature and chemical processing methods on MPs levels. This study was conducted using MPs samples derived from facial soap products. Then, it is processed by heating at 100°C for 10 minutes and dissolved with KOH solution.[9] It was found that there were changes in MPs particles after being given heat exposure. Temperature treatment on MPs will cause polymer degradation.[14.] Other research by Claessens et al. (2013) found that polystyrene MPs undergo clumping and melting at a temperature of 100°C. Research by Trinity College Dublin says that the release of MPs is temperature- dependent, i.e., the release of MPs increases with an increase in temperature. MPs particles were found in plastic milk bottles filled with water with a temperature of 95°C.[15]

Another study conducted by Iwalaye et al. (2020) explained that there was an increase in MPs ingested by sea cucumbers at high water temperatures of 28°C. This research method was carried out by providing MPs exposure to each fish tank and differentiating by different temperatures (19°C, 24°C, 28°C, and 32°C). MPs are taken through intestinal and fecal samples to calculate ingestion and retention from sea cucumbers. It was found that with an increase in temperature, there was an increase in ingestion and retention of MPs particles. This occurs due to low temperatures sea cucumbers tend to hibernate, so they rarely eat. Conversely, at high temperatures sea cucumbers tend to be more active in eating so that the concentration of digested MPs is also higher.[16]

The results of measuring the diameter of MPs particles obtained the smallest result is 0.01 mm and the largest is 0.68 mm. The smallest MPs particle shape is fragments and the largest MPs particle shape is filaments. According to Lippiat et al. (2013) plastics are divided by size, namely mega (>100 cm), macro (>2.5-100 cm), meso (>5-25 mm), micro (1-5000 µm), and nano (<1 µm). Based on these criteria, the particles found in this study are MPs.[17.]

The results of descriptive analysis of particle shapes in 4 groups of breast milk samples showed that the mean and standard deviation of the most MPs forms were fragments found in the 4°C temperature group with levels of 8.43 ± 9.06 particles/5 cc. The shape of fragments in the control group with levels of 1.28 ± 1.41 particles/5 cc is the least. The group with the most filaments was the temperature group of 40°C and the least in the control group with levels of 0.15 ± 0.42 particles/5 cc. There are several forms of MPs, namely fragments that have characteristics, namely round, angular, and subangular, pellets, namely cylinders, discs, round, flat, and oval. The second form is filament or fiber which has the characteristics of irregular, blunt angles, and granules.

Micro-FTIR is a check for the identification of MPs based on waves.[18] The polymer wavelength in micro-FTIR examination is matched to the polymer wavelength so that the type of polymer can be known. In the analysis of wavelength peaks contained in the results of micro-

FTIR, 19 polymers were found and 16 of them were MPs. Based on the results of the micro-FTIR, can confirm that there are MPs particles in breast milk. The most MPs particles found were nylon as much as 56.67%. Research by Liu et al. (2023) says that the most common types of MPs found in breast milk bags are PE, PET, and nylon.[7] Nylon is a synthetic polymer composed of polyamide, a thermoplastic material that can be processed into various types of objects. Nylon polymers can be mixed with a wide variety of additives to form different properties and can be used on different types of fabrics and fibers, molds for automobiles, electrical appliances, food packaging, and others.[19]

CONCLUSION AND SUGGESTION

Based on the results of research that has been done, it was found that there was no significant difference in MPs particle levels against the difference in breast milk processing temperature in storage bags. However, there were significant differences between the control group and the study group.

ACKNOWLEDGEMENT

The author wishes to express sincere gratitude to Puskesmas Mulyorejo for providing the facility and support in conducting this research. The author also extends thanks to all individuals and parties who contributed and were involved in the completion of this study.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest related to the conduct or publication of this research.

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