

# The Effect of Breast Milk Storage Container on the Amount of Probiotic Microbiota in Breast Milk

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## Abstract

Developing babies require important bacteria from breast milk, which impact gut microbiota in adulthood and lifelong health. Breast milk also contains hundreds to thousands of different microbiomes. These microbiomes prevent infection, inflammation, organ growth, healthy microbial colonization, and aid immune maturation. The type of storage container material used influences bacterial colonization of breast milk. The aim of this study was to analyze the effect of storage containers on breast milk probiotic microbiota. The type of research is a true experiment with a pretest and posttest control group design. The probiotic microbiota studied is Lactic Acid Bacteria (LAB). The examination of the total colonization of lactic acid bacteria was carried out using the Quebec Colony Counter Unit. Data were analyzed by paired sample T-test. The results showed that the average number of Lactic Acid Bacteria colonies stored in glass bottle is greater than the number of LAB colonies stored in plastic bag (95x10<sup>6</sup> CFU/ml vs 77x10<sup>6</sup> CFU/ml). However, statistical tests showed no significant difference between the number of BAL colonies of breast milk stored in plastic bags and breast milk stored in glass bottles.

Keywords: Breast milk, Storage container, Probiotic Microbiota.

## Introduction

The bioactive components of breast milk are microorganisms. In the middle of the last century, the presence of breast milk was first recognized when studying the possibility of transmitting infections through breastfeeding. Several studies have shown that commensal, mutualistic, or possibly probiotic bacteria present in breast milk are healthy and their impact on the health of mother and baby. Several strains of the species *Lactobacillus salivarius*, *Lactobacillus fermentum*, *Lactobacillus gasseri*, *Bifidobacterium breve*, *Bifidobacterium adolescentis*, and *Bifidobacterium longum* subsp. *Infantis* is a microorganism found in milk. In breast milk samples taken one month after vaginal delivery, there were 0.1 to 3% *Bifidobacterium* and 0.1 to 0.3% *Lactobacillus*. Healthy breast milk contains 10<sup>3</sup>-10<sup>5</sup> cfu/ml of live bacteria, according to studies using culture.(1)

Developing babies require important bacteria from breast milk, which impact gut microbiota in adulthood and lifelong health. Breast milk also contains hundreds to thousands of different bioactive molecules. These bioactive molecules prevent infection, inflammation, organ growth, healthy microbial colonization, and aid immune maturation. Oligosaccharides, a part of breast milk, are responsible for promoting the development of several microbes. In addition, antimicrobial peptides, lactoferrin, and lysozyme can stop the development of pathogenic bacteria.(1)

The neonatal period is a critical phase for microbial colonization of the digestive tract. Initial microbial colonization is influenced by one of the factors involved in breast milk storage. Globally, the complex and dynamic composition of breast milk promotes the healthy growth and development of babies. Therefore, WHO recommends exclusive breastfeeding for the first 6 months. It is known that a large number of microbes colonize the intestines of babies in the first days and weeks of life.(2)(3)

Babies consume 800 milliliters of breast milk per day, which contains  $1 \times 10^5$  and  $1 \times 10^7$  bacteria per day. These bacteria are expected to survive in the gastrointestinal tract and enter the baby's intestines because of the fast transit time and relatively low gastric pH. Several previous studies which used standard culture techniques, comparing the microbiota of formula-fed and breast-fed infants during the first 3 weeks, they found an increase in *Lactobacillus* and *Bifidobacteria* species of 10<sup>3</sup>-10<sup>4</sup> cells/ml and 10<sup>2</sup>-10<sup>5</sup> cells/ml. The study conducted by Yoshioka et al. using

standard culture techniques compared the microbiota of breast-fed and formula-fed babies for the first three weeks. Babies who are fed formula milk have microbiota patterns such as enterobacteria and enterococci. The results show that breastfeeding creates a favorable intestinal environment for the growth of bifidobacteria.(1)

The many benefits of breast milk for a baby's life make breastfeeding very important and a priority for babies. However, in reality, exclusive breastfeeding is not optimal in society, especially for working mothers. Working mothers feel that there are many obstacles in providing exclusive breastfeeding because they do not know how to store and handle breast milk. Improper use of breast milk storage containers can reduce the quality of the breast milk provided.(1)

The type of storage container material used influences bacterial colonization of breast milk. Pranathami (2020) found that the average number of bacteria in plastic bags was the highest, namely  $5.4 \times 10^3$  CFU/ml, the average number of bacteria in plastic bottles was  $4.4 \times 10^3$  CFU/ml, and the number of bacteria in glass bottles was  $4.4 \times 10^3$  CFU/ml. .CFU/ml. is  $4.3 \times 10^3$  CFU/ml. This shows that these materials function well as ASIP storage containers because they are not contaminated by external bacteria.(4)

Studies on the effect of storage containers on the colonization of lactic acid bacteria are still limited. Even though, lactic acid bacteria is an important bacteria contained in breast milk and the quantity must be maintained. Lactic Acid Bacteria is able to inhibit growth of pathogenic bacteria

The aim of this study was to analyze the effect of storage containers on breast milk probiotic microbiota. This research is useful as a reference in selecting breast milk storage containers so that babies can consume quality breast milk in terms of the amount of microbiota.

## Method

Method should be structured as follows:

### *1. Research design*

The type of research is a true experiment with a pretest and posttest control group design. The dependent variable of this research is breast milk probiotic microbiota colonization and the independent variables are storage container for breast milk. The

probiotic microbiota studied is Lactic Acid Bacteria (LAB).

## *2. Setting and samples*

The population of this study were breastfeeding mothers who were in the working area of the Air Dingin Health Center. The sample consisted of 5 people taken using consecutive sampling technique. The samples taken were breast milk that met the inclusion criteria, namely a history of normal delivery, normal body mass index and no metabolic disease

## *3. Intervention (applies to experimental studies)*

This study used a type of breastmilk container consisting of A (glass bottle) and B (plastic bag) which was stored at room temperature with three repetitions. The process of collecting/expressing breast milk begins by minimizing cross-contamination of bacteria from mothers into breast milk by asking respondents to shower, wash their hands with antiseptic soap first, cut their nails, and use a mask. The breastmilk in the pump bottle is then divided into 2 each breast milk storage containers made from glass bottles and plastic bags and labeled/treated. The breastmilk sample is then analyzed for total bacterial colonies in the laboratory.

## *4. Measurement and data collection*

10 ml of breast milk was taken from each breastfeeding mother. Then, 5 ml of breast milk was put into a sterile plastic bag and 5 ml was put into a sterile glass bottle. Then, each sample was stored for 6 hours in a cooler bag. After 6 hours, the examination of the total colonization of lactic acid bacteria was carried out using the Quebec Colony Counter Unit. Bacterial colonization examination was carried out in the laboratory of the Faculty of Animal Husbandry, Andalas University.

## *5. Data analysis;*

Data were analyzed by paired sample T-test

## Results

**Table 1**  
**Differences in the Mean Number of Lactic Acid Bacteria Colonies in Breast Milk Based on Storage Containers**

The Type Of Container	Mean (CFU/ml)	P
Glass Bottle	95 x 10 <sup>6</sup>	0,38
Plastic Bags	77 x 10 <sup>6</sup>	

Table 1 shows that the average number of Lactic Acid Bacteria colonies stored in glass bottles was greater than the number of LAB colonies stored in plastic bags (95x10<sup>6</sup> CFU/ml vs 77x10<sup>6</sup> CFU/ml). However, statistical tests showed there was no significant difference between the number of LAB colonies in breast milk stored in plastic bags and breast milk stored in glass bottles (p value=0.38).

## Discussion

The results showed that the average number of Lactic Acid Bacteria colonies stored in glass bottle is greater than the number of LAB colonies stored in plastic bag (95x10<sup>6</sup> CFU/ml vs 77x10<sup>6</sup> CFU/ml). However, statistical tests showed no significant difference between the number of BAL colonies of breast milk stored in plastic bags and breast milk stored in glass bottles.

The results of this research are in line with research by Pranatami (2020) showing that variations in container have no effect on the total bacterial colonies in breast milk with a p value of 0.246. The highest number of bacteria was in plastic storage bags (average 5.4x 10<sup>3</sup> CFU/ml). However, this study did not examine specific types of bacteria.(4)

The results shows that these materials (glass bottle and plastic bag) show good results as ASIP storage containers because they cannot be contaminated with bacteria from the outside. This can be caused by the type of breastmilk container used is in accordance with Standard Operating Procedures. Plastic bags made from polyethylene (PE) is plastic packaging commonly used to package food products. Based on the results of the research above, it can be seen that storing breastmilk in plastic bags has a higher average total bacterial colony than in glass bottles because polyethylene has a slightly higher oxygen permeability which results in the availability of sufficient oxygen to stimulate the growth of aerobic microbes because it is easy to absorb. oxygen from outside. Glass and polypropylene materials have lower water vapor permeability and

can prevent contact between the material and oxygen, making it better able to prevent microorganism contamination.

Mother's milk is one of the ingredients It is very easy for bacteria to grow because the nutritional composition of carbohydrates, vitamins, minerals, protein and fat is quite high which is very beneficial for the growth of microorganisms. Bacteria contained in breast milk include *Lactobacillus* spp., *Staphylococcus* spp., *Bacillus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Enterococcus* spp. Breast milk contains probiotic bacteria which are widely known to belong to the Lactic Acid Bacteria (LAB) group. LAB has the benefit of improving the quality and safety of food ingredients through natural inhibition of pathogenic microorganisms.(5)

#### Limitation

The drawback of this research is that it did not examine bacterial colonies in plastic bottle containers and did not relate this to temperature and storage time

#### Conclusion

There was no effect of the type of storage container on LAB colonization in breast milk, although the average LAB colonization in breast milk stored in glass containers was higher than the average LAB colonization in breast milk stored in plastic containers.

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#### **Author contribution**

Contributions in writing this article were carried out by the main author and member writers, we work together in making this article according to the template provided

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