

# Processing matters

As a neonatologist, NICU nurse, nutritionist, lactation consultant, or related medical professional, you may be making purchasing decisions about human milk products for your hospital. Understanding the impact of different processing methods on the bioactivity of human milk is an important aspect of the decision-making process.



# More Than Food

Human milk includes species specific bioavailable components that are just as important as the macronutrients critical to optimal newborn development. These components play major roles in disease protection and support of a healthy microbiome. When medically vulnerable infants don't have access to their mother's milk, the goal is to provide human milk that has been processed to eliminate pathogens while maximizing the retention of nutrients and unique bioactive factors. These bioactive components include, but are not limited to:

- Lactoferrin – A multifunctional protein that facilitates iron absorption and inhibits bacterial growth; present in quantities 100x greater than found in bovine milk.
- Lysozyme – An antibacterial protein that kills gram-positive and gram-negative bacteria; present in quantities 3000x greater than found in bovine milk.
- Immunoglobulins – Antibodies custom to pathogens in the maternal environment; one in particular, secretory immunoglobulin A (sIgA), is present in quantities 4000x greater than found in bovine milk, and constitutes 90% of the total immunoglobulins in human milk.
- Human Milk Oligosaccharides (HMOs) – HMOs are the third most abundant factor in human milk yet they are indigestible by infants. These short chain sugars serve many functions in the intestinal tract, including pathogen and toxin binding, enrichment of beneficial gut bifidobacterial, immune system support, and enhancement of the intestinal epithelial barrier function.
- Lysine– An amino acid that plays an important role in the production of carnitine which is necessary for converting fat into energy.

## Overview of Milk Processing Methods

### **Holder Pasteurization and Vat Pasteurization for Frozen Human Milk**

Globally, Holder pasteurization is widely used by milk banks to inactivate bacteria and viruses. In this low-heat method of processing, bottled milk is warmed to 62.5°C, held for 30 minutes, rapidly cooled, and then frozen until use. While there are no changes in the overall macronutrient profile of pasteurized human milk, retention of bioactive factors varies. Nonprofit milk banks accredited by the Human Milk Banking Association of North America (HMBANA) only use Holder pasteurization. Nearly all research regarding the efficacy of donor milk has used milk processed by Holder pasteurization. Vat pasteurization, used by some for-profit human milk derived product manufacturers, is a larger scale version Holder pasteurization.

### **Retort Processing for Shelf-Stable Human Milk**

Retort processing, an older technology (canning), is currently being used by some for-profit corporations to produce a shelf-stable human milk product. Retort processing (shelf-stable human milk) uses high temperatures (115°C to 145°C) under pressure for several minutes to sterilize human milk. While this method is economical and allows for the efficient processing of large quantities of milk from hundreds of donors, emerging research suggests that retort processing significantly reduces several key bioactive components. To date, there is no data regarding clinical outcomes in the recipients of shelf stable donor milk.

***All HMBANA accredited non-profit milk banks use the Holder Method of Pasteurization. This small batch, low temperature process ensures safety while retaining bioactivity.***

## Differences in Holder/Vat Pasteurization Versus Shelf Stable Human Milk (Retort)

Attributes	Meredith-Dennis (2017) <sup>7</sup>	Lima (2017) <sup>8</sup>	Lima (2018) <sup>9</sup>	Liang (2022) <sup>10</sup>
Types of Milk	Holder; shelf-stable	Raw; Holder; shelf-stable	Raw; Holder; shelf-stable	Raw (previously frozen), thawed and refrozen, thawed, homogenized and refrozen, Vat, Retort, Retort plus homogenization, Ultra High Temperature, Ultra High Temperature plus homogenization
Sample Size	N = 3 per milk type, each type received from a different milk bank	N = 36 total from the same pool 12 samples raw, 12 Holder, 12 shelf stable	N = 36 total from the same pool 12 samples raw, 12 Holder, 12 shelf stable	N=18 total samples of 7 different types from the same pool. The number of donors that contributed to the pool was not reported
Study Design	Cross-sectional (each milk type was from different donors and had a different pool size)	Cross-over (each milk type was from the same combined milk pool of 60 donors)	Cross-over (each milk type was from the same combined milk pool of 60 donors)	**Cross-over (each milk type was taken from the same pool)
Lactoferrin	*Higher in Holder vs shelf-stable	Not measured	Not measured	Vat retained 35% shelf stable (retort) retained 16%
Immunoglobulins	*Higher IgM and IgG in Holder vs shelf-stable	Holder retained 87% sIgA; shelf-stable retained 11% sIgA	Not measured	Vat slightly decreased IgA and IgM but not IgG
Lysozyme	*No difference between Holder vs. shelf-stable	Holder retained 54%; shelf-stable retained 0%	Not measured	No significant changes after any of the studied processing methods
HMO	*Higher in Holder vs shelf-stable	Not measured	Not measured	Not measured
Lysine	Not measured	Not measured	Raw= 0.85 mg/100 mL Holder=0.77 mg/100 mL shelf-stable=0.68 mg/100 m	Not measured
Thiamine	Not measured	Not measured	Raw= 0.24 mg/L Holder=0.26 mg/L shelf-stable=0.14 mg/L; p<0.01	Not measured
Bacteria Levels	Not measured	B. Cereus detected in 3 Holder samples; no bacteria detected in shelf-stable.	Not measured	Not measured
Other	Holder was higher in protein, fat, caseins ( $\alpha$ , $\beta$ , $\kappa$ ), $\alpha$ -1-antitrypsin, $\alpha$ -lactalbumin, and osteopontin, likely due to the fact that Holder milk was from preterm donors.	Not measured	Not measured	Growth Factors: No change in immunoreactivity of VEGF or TGF $\beta$ 2 with Vat Pasteurization. 95% reduction in VEGF and 92-99% reduction in TGF $\beta$ 2 in shelf stable (retort) samples.
Conclusions	Differences in processing, pooling of milk, and stage of lactation may contribute to differences in nutrient and bioactive composition, warranting further research.	Significant loss of bioactive proteins in shelf-stable milk compared to Holder. Holder requires post pasteurization testing for B. Cereus. HMBANA milk banks do not dispense milk with B. Cereus or other pathogens detected.	Macronutrient content is relatively unaffected by processing. Lysine and thiamine were significantly decreased in shelf stable milk but not by Holder. Thiamine losses are clinically significant and fortification may be necessary.	Overall, Vat pasteurization preserved more of the bioactive proteins compared with Ultra High Heat or Retort Sterilization. Therefore, human milk processors should consider the impact of processing methods on key bioactive proteins in human milk.

*\*This study was a cross-sectional study, so difference in composition may be attributed to different donors and different stages of lactation; therefore, the scale of differences was not assessed due to lack of control.*

*\*\* This study was funded by a for-profit human milk derived products manufacturer*

There is a high loss of bioactive factors in shelf-stable human milk which may translate into different health outcomes in the medically fragile infant. More research is warranted before use of retort processed milk can be recommended for fragile infants. Several promising food science technologies are being investigated for use with human milk. These include high pressure processing, ultraviolet radiation, and high-temperature short-time processing. At this time, fundamental knowledge is lacking and extensive research is still required before using these processing methods with human milk. Meanwhile, non-profit milk banks, such as those within the Human Milk Banking Association of North America's network, continue to use Holder pasteurization for human milk.

Mid-Atlantic Mothers' Milk Bank ("Three Rivers") is a non-profit milk bank that serves Pennsylvania, West Virginia, and surrounding Mid-Atlantic states. Our milk bank opened in January 2016 with generous funding from our region's foundations, health systems, and corporations to improve the health outcomes of our tiniest, most vulnerable residents.

In addition to providing donor milk, Mid-Atlantic Mothers' Milk Bank offers educational programming for clinicians and programming for families experiencing infant loss.



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#### References

1. Lönnerdal B. (2013) Bioactive proteins in breast milk. *J Paediatr Child Health*, 49.
2. Hettinga K et al. (2011) The host defense proteome of human and bovine milk. *PLOS One*, 4(6).
3. Kunz C et al. (2002) Oligosaccharides in human milk: Structural, functional, and metabolic aspects. *Annu Rev Nutr*, 20.
4. PATH. (2013) Strengthening human milk banking: A global implementation framework. [http://www.path.org/publications/files/MCHN\\_strengthen\\_hmb\\_frame\\_Jan2016.pdf](http://www.path.org/publications/files/MCHN_strengthen_hmb_frame_Jan2016.pdf)
5. Ewaschuk JB et al. (2011) Effect of pasteurization on selected immune components of donated human breast milk. *J of Perinatol*, 31(9).
6. Peila C et al. (2017) Human milk processing: A systematic review of innovative techniques to ensure the safety and quality of donor milk. *J Pediatr Gastroenterol Nutr*, 64(3).
7. Meredith-Dennis L et al. (2017) Composition and variation of macronutrients, immune proteins, and human milk oligosaccharides in human milk from nonprofit and commercial milk banks. *J Human Lact*, doi: 10.1177/0890334417710635
8. Lima H et al. (2017) Bacteria and bioactivity in Holder pasteurized and shelf-stable human milk products. *Curr Dev Nutr*, doi: <https://doi.org/10.3945/cdn.117.001438>
9. Lima H et al. (2018) Nutritional comparison of raw, Holder pasteurized, and shelf-stable human milk products. *J. Pediatr Gastroenterol Nutr*, doi: Jul 30. doi: 10.1097/MPG.0000000000002094
10. Liang N et al. (2022) Structural and functional changes of bioactive proteins in donor human milk treated by vat-pasteurization, retort sterilization, ultra-high-temperature sterilization, freeze-thawing and homogenization. *Frontiers in Nutrition*, 9. doi=10.3389/fnut.2022.926814 <https://www.frontiersin.org/articles/10.3389/fnut.2022.926814>