

Effect of the PiAstra Benchtop Flash-Heating Pasteurizer on Immune Factors of Donor Human Milk

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Abstract

Introduction: PiAstra is a simulated flash-heat (FH) pasteurization temperature monitoring system designed using Raspberry Pi technology for the pasteurization of human milk. This study analyzed the effect of the PiAstra FH method on human milk immune components (immunoglobulin A [IgA] and lactoferrin activity).

Methods: Donor milk samples ($N=45$) were obtained from a human milk bank, and pasteurized. Concentrations of IgA and lactoferrin activity were compared to their unpasteurized controls using the Student's t test.

Results: The PiAstra FH method retained 34.2% of IgA ($p<0.0001$) and 40.4% of lactoferrin activity ($p<0.0001$) when compared to unpasteurized controls. The retention of IgA by the PiAstra is similar to previous FH studies, while retention of lactoferrin activity was higher than previous FH studies.

Discussion: The high-technology, low-cost PiAstra system, which is able to retain vital immune components of human milk, provides safe donor milk for low-resourced settings. This enables the use of pasteurized donor milk when human milk is not available, potentially saving vulnerable infant lives.

Keywords: human milk banking, pasteurization, flash-heat

Introduction

THE BENEFITS THAT HUMAN MILK provides go beyond optimum nutrition to the provision of digestive enzymes, immune factors, hormones, growth factors, and many other bioactive components.^{1,2} The immune protection provided by human milk is especially important for vulnerable infants in resource-limited settings—such as preterm, low-birthweight, HIV-exposed, or orphaned infants. These infants are at increased risk of infectious diseases, malnutrition, and mortality, and the main cause of this is often the absence of safe and adequate nutrition. Human milk contains a multitude of immunomodulatory compounds, including antibodies (IgMs, IgGs, IgA), oligosaccharides, saturated and unsaturated fatty acids, soluble receptors (e.g., CD14), cytokines and chemokines, prebiotics, immune cells, and antibacterial proteins/peptides (e.g., lactoferrin and lysozyme).^{1,3} Lactoferrin has antimicrobial activity against a large number of bacteria, fungi, and viruses; anticancer and anti-inflammatory properties; and is one of the most abundant human milk proteins.^{1,3,4} Approximately 90% of total immunoglobulins (IgAs) in milk are made up of secretory IgA, which ensures that antibodies to specific antigens that the mother encounters are passed on to the infant.^{1,4}

Although South Africa has high breastfeeding initiation rates, mixed feeding and early cessation of breastfeeding are common.⁵ South Africa falls short of the global exclusive breastfeeding (EBF) targets, however, these rates have been increasing with 31.6% of infants recently being estimated to be EBF for the first 6 months.⁶ This improvement can be attributed, in part, to the recommendation by the South African government at the 2011 National Department of Health Breastfeeding Consultative Meeting to cease the free provision of formula milk at hospitals and clinics, and encourage EBF for all mothers, regardless of HIV status.⁷ Further recommendation was that all hospitals with neonatal intensive care units (NICUs) establish donor human milk banks (also known as human milk banks [HMBs]) to provide donor human milk for specific cases where the mother's own milk was not available due to mother's severe illness, maternal death, or the mother's inability to breastfeed.

These HMBs are able to make safe human milk (donor human milk) available to those infants most in need and have demonstrated improved infant survival and health, as well as reduced cost burden on the healthcare system.^{8–11} These HMBs use national guidelines to screen donors and pasteurize human milk accordingly.^{11,12}

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The ability to pasteurize human milk is the central tenet of being able to operationalize HMBs. In the developed world, HMBs typically utilize the Holder pasteurization method using commercial pasteurizers, which can range from \$10,000 to \$30,000. For low-income countries, which have a greater need for donor milk as more infants are at increased vulnerability, innovative low-cost pasteurization methods are necessary to initiate HMBs. In addition, several less developed countries have large numbers of orphaned and abandoned infants unable to access human milk, which is vital in such vulnerable communities. Concerns about wet nursing and sharing unpasteurized human milk have made it difficult to meet these orphans' needs and since traditional pasteurization processes used by HMBs are expensive, providing them with pasteurized donor milk is prohibitive. In addition, smaller hospitals in rural settings are also unable to access donor milk for low-birthweight babies in NICUs due to accessibility and financial challenges.

Flash-heat (FH) pasteurization is a low-technology, inexpensive method of human milk pasteurization designed to imitate commercial high-temperature short-time heat treatments (HTSTs), which typically heat to 72°C for 15 seconds. This process is impossible in developing countries without high-technology equipment. In the FH method, 50–100 mL of milk is placed in an uncovered 450-mL glass food jar, which is then placed in ~450 mL of water in a 1–2-L aluminum pan. The water and milk are heated simultaneously using an electric hot plate until the water reaches 100°C and is at a rolling boil. The human milk jar is removed immediately from the water bath and allowed to cool before being used or it is cooled briskly in an ice bath and frozen if it is to be stored for future use. Before the advent of prescribing antiretroviral drugs during breastfeeding, several South African hospitals had routinely used the flash-heating method for HIV-positive mothers. They flash heated their own milk to destroy HIV before feeding the human milk to their infants. King Edward Hospital (Durban, South Africa) has used this method for pasteurizing donor milk for use in the NICU.^{13,14}

HTST pasteurization methods are apparently superior as they kill cytomegalovirus and heat-susceptible, nonspore-forming bacteria, with limited decrease in vitamins, lactoferrin, total IgA concentrations, or secretory IgA activity, therefore making FH an ideal method for low-income settings.¹⁵ Flash-heating has demonstrated inactivation of both cell-associated and cell-free HIV-1,^{15–17} without diminishing the bacteriostatic potential of human milk.¹⁸ Previous studies have shown that FH typically resulted in a 20% decrease in total IgA and 33% of total IgG in human milk from HIV-infected mothers.¹⁸ Comparable reductions were seen in anti-pneumococcal polysaccharide, anti-HIV-1 gp120 IgG, and anti-poliovirus IgA. Despite anti-poliovirus IgA being most affected, FH retained 66% of the unheated antigen binding ability.¹⁸

Previously, a low-cost temperature monitoring system was designed by Chaudhri et al.,¹⁴ which made use of FoneAstra, a low-cost, mobile phone-based network sensing system developed by the Department of Computer Science and Engineering at the University of Washington (UWCSE), in partnership with Program for Appropriate Technology in Health (PATH). FoneAstra was designed to monitor the milk temperature during flash-heating and deliver constant

audiovisual feedback to staff to direct the pasteurization process.

The FoneAstra system is a simulated FH pasteurization system, with a larger volume of milk (120 mL) being heated in a jar. This is unlike the typical system of pumping the milk through fine capillary tubes to ensure that all parts of the milk are evenly heated to 72°C for only 15 seconds. In the FoneAstra and other simulated systems that use larger volumes, it is difficult to keep the milk at 72°C for only 15 seconds because of the greater latent heat that is present in these larger volumes of milk. Temperatures therefore typically reach 72°C for about 30 seconds.¹⁹ It is therefore important when using a simulated system to ensure that, while potentially pathogenic bacteria, fungi, and viruses are sufficiently inactivated, the beneficial protective/immune components of the human milk are still retained.

When comparing the FoneAstra system to the Holder pasteurization system, concentrations of human milk oligosaccharides,²⁰ interleukin-8 (IL-8) and IL-10, and lysozyme activity¹⁹ remained similar to those for Holder pasteurization. However, only 38.6% of lactoferrin activity and 25.2% of IgA (compared with 71.1% and 78.9% with Holder) were retained with FoneAstra.¹⁹

One of the possible reasons for this higher than expected destruction of immune factors may be that the FoneAstra system's temperature probe was placed in water, which may not be a reliable proxy of what occurs in the human milk samples and thus could have resulted in the milk being subjected to temperatures higher than 72°C. After experimentation, it was decided to use a temperature probe in full-fat cow's milk, which served as a better proxy.

Since the FoneAstra system, which is similar to the PiAstra system being tested, showed very little negative impact on oligosaccharides, IL-8, IL-10, and lysozyme, it was assumed that the PiAstra system would likely have a similar impact and that only the two immune components (lactoferrin and IgA) that were previously most adversely affected should be tested.^{19,20} This study therefore investigated the effect of the PiAstra FH simulation system on lactoferrin and IgA in human milk. In addition, the study tested the efficacy of the pasteurization system to eliminate bacteria, a key objective of pasteurization in HMBs.

Materials and Methods

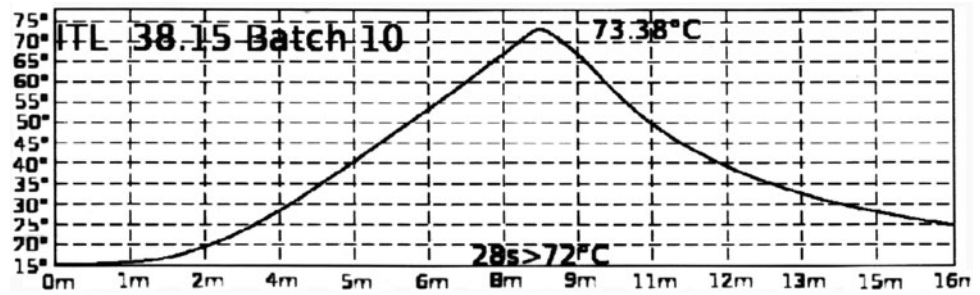
Development of the PiAstra pasteurization system

The FoneAstra was modified to make it more cost-effective by using the shelf modular Raspberry Pi technology



FIG. 1. PiAstra equipment and FH human milk treatment setup. FH, flash-heat.

FIG. 2. Typical PiAstra FH temperature curve. The X-axis shows time in seconds, while the Y-axis shows degrees centigrade. Each graph includes the batch number, peak temperature reached, and length of time that the sample temperature was over 72°C. FH, flash-heat.



as well as a touch screen for input and output data in place of a mobile phone. This modified pasteurizer is now called the PiAstra—Figure 1 shows the PiAstra equipment setup. Standard research and development procedures were used to develop the device and, in particular, the temperature at which the milk would be removed from the water bath. In the initial work, we compared temperatures of 71.5 and 72°C, respectively, and found that both temperatures were effective in inactivating heat-susceptible bacteria and that the retention of IgA was higher at the lower setting (unpublished data). Therefore, the system was set for removal of milk from the water bath when the milk reached 71.5°C. At this temperature, the milk was heated above 72°C for a mean time of 30.7 seconds, whereas setting the pasteurizer at 72°C resulted in the milk being exposed to 72°C for a mean time of 40.2 seconds. Figure 2 shows the temperature curve for a typical PiAstra pasteurization cycle.

Study samples

Forty-five samples (250 mL) of frozen anonymous donor human milk were obtained from an HMB. As per standard regulations, donors were healthy, HIV-negative women who were screened for absence of any lifestyle risks. Of the 45 samples, 31% (14 of 45) were from mothers with preterm deliveries. Donors were mostly Caucasian (82%) or African (13%).

Human milk pasteurization

Donor human milk samples were defrosted and 2 × 1 mL aliquots of each donor's human milk were frozen to serve as unpasteurized controls. The remaining milk of each donor was pasteurized using the PiAstra system (FH method). On completion of pasteurization, 2 × 1 mL samples were taken out of each bottle and frozen. Therefore, from each donor human milk sample, there were two aliquots: unpasteurized human milk and human milk pasteurized using the PiAstra method. These aliquots were frozen at -70°C for later analysis. Unpasteurized samples are referred to as "Control" samples throughout the remainder of this document, while "PiAstra" represents the FH samples. The Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE420/17) granted ethical approval for the study.

Assays performed after pasteurization

Microbiology assay. Frozen samples were transported to the South African National Health Laboratory Services lab-

oratory, defrosted, gently mixed, and a 100 μ L aliquot of human milk sample was spread plated (in duplicate) under sterile conditions evenly onto Columbia agar plates containing 5% horse blood (manufactured by the NHLS). Inoculated plates were allowed to dry on a clean bench facing upward for 1 hour before incubation at 37°C in a 5% CO₂ incubator for 24 hours. After incubation, the plates were examined for the presence of microbial colonies. As per international standards,²¹ there should be less than 10 colony-forming units per milliliter (CFU/mL) of pasteurized human milk for the sample to be considered acceptable. Since the inoculum was only 100 μ L, any sample that presented microbial growth was considered contaminated.

Antibody assay. IgA was measured using the Abcam human IgA ELISA kit as per the manufacturer's recommendations. Briefly, all samples were assayed in duplicate. Samples were thawed completely and vortexed thoroughly before being tested. A 1:20,000 dilution was used for the IgA assays as per the manufacturer's recommendation.

Lactoferrin assay. The lactoferrin assay was performed as reported previously.¹⁹ Briefly, 5 mm sample wells were created in the solidified Kundrat agar (Sigma-Aldrich, South Africa) using a sterile Pasteur pipette. Each breast milk sample was assayed in duplicate, where 25 μ L of human milk sample was placed into the well, followed by incubation at 42°C for 20 hours. All samples were analyzed in duplicate and zones of endospore germination inhibition were determined after incubation using a digital Vernier caliper (Marshal Tools, South Africa). Using authentic bovine lactoferrin as calibration standard (Sigma-Aldrich), inhibition zones were converted to μ g of lactoferrin per mL of human milk.

TABLE 1. CONCENTRATION VALUES OF IMMUNE COMPONENTS AND THEIR PERCENTAGE RETENTION WITH THE PiASTRA PASTEURIZATION METHOD

	Control (\pm SD)	PiAstra (\pm SD)	Percentage retention (\pm SD)
Lactoferrin (μ g/mL)	115.9 (233.8)	41.6 (94.02) ^a	40.4% (17.39)
IgA (μ g/mL)	380.1 (119.2)	139.3 (112.4) ^a	34.2% (21.1)

Values listed are means (\pm SDs).

^a $p < 0.0001$.

IgA, immunoglobulin A; SD, standard deviation.

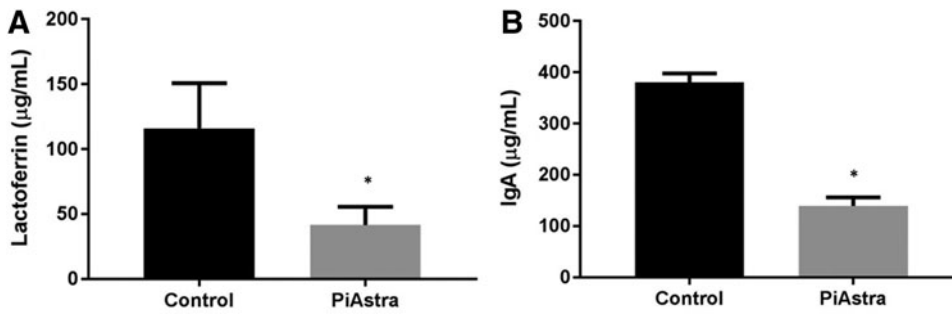


FIG. 3. Effect of simulated FH on the retention of immune components expressed as percentage retention. (A) Lactoferrin; (B) IgA. Bars represent means \pm SEM. FH, flash-heat; IgA, immunoglobulin A. * $p < 0.0001$.

Statistics

Differences between group pairs were analyzed with a Student's *t* test. Outliers were identified and removed from the analysis using the Rout method, establishing a maximum false discovery rate of 1% ($Q = 1$). All analyses were performed using GraphPad Prism version 7 (GraphPad Software, San Diego, CA). Data in bar graphs are presented as medians with 95% confidence intervals. Findings were assumed statistically significant at $p < 0.05$.

Results

Previous studies conducted in our laboratory have shown that prepasteurized milk (i.e., control milk), using the identical testing protocol, typically yielded $>10^5$ CFU/mL of microbial growth.²² None of the 45 PiAstra FH samples gave rise to colonies after incubation, and therefore, these FH-treated human milk samples met the prescribed quality threshold of less than 10 CFU/mL.²¹

The analysis of IgA and lactoferrin in unpasteurized control samples and PiAstra FH samples demonstrated an expected destruction of both immune factors (Table 1). The mean lactoferrin concentration in control samples was 115.9 $\mu\text{g/mL}$, while the PiAstra samples showed a mean of 41.6 $\mu\text{g/mL}$, with retention of 40.4% (Fig. 3A). Mean IgA concentrations in control samples were 380.1 and 139.3 $\mu\text{g/mL}$ in PiAstra samples, with retention of 34.2% (Fig. 3B).

Discussion

There is a need for HMBs in low-income countries to provide safe donor human milk using more cost-effective and low-technology methods, without compromising on the nutritional value of the pasteurized human milk. Several studies have previously evaluated the impact of pasteurization on human milk immune components. However, little research has focused on evaluating flash-heating. Holder pasteurization has been shown to reduce the function of immunoreactive proteins such as IgA, lysozyme, and lactoferrin by up to 80%.^{23–28} Previous studies have found similar results with flash-heating on IgA^{28,29} and lactoferrin.^{15,28} One flash-heating study, which showed a 66% IgA retention,¹⁸ pasteurized 50 mL milk volumes and therefore may not be comparable, as the heating dynamics in such different volumes of milk may be substantial. Our earlier testing FoneAstra-simulated flash-heating system showed 25.2% and 38.6% retention of IgA and lactoferrin, respectively. In this study, in addition to achieving the required microbiological quality threshold value of <10 CFU/mL, the PiAstra system showed similar retention

of lactoferrin, while showing better retention of IgA. While both the FoneAstra and the PiAstra systems show less retention of immune components of human milk compared with traditional Holder pasteurization, not all immune factors are adversely affected and it appears that the majority would be preserved. While pasteurization of human milk comes at a cost of diminished IgA and lactoferrin, this needs to be weighed against a system that provides a cost-effective, mobile, water-efficient technology for effectively destroying bacteria with minimal destruction of immune components.

The availability of a simple, user-friendly, low-cost (\sim \\$900), benchtop, mobile device, simulating flash pasteurization, provides opportunities for communities and NICUs to provide safe donor human milk to orphaned/abandoned infants and low-birthweight infants in low-resourced settings and rural hospitals.

Conclusion

In conclusion, the low-cost but high-technology PiAstra pasteurizing device makes it possible to promote the use of pasteurized donor milk when human milk is not available, thus promoting breastfeeding in the general population and improving outcomes for vulnerable infants.

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Authors' Contributions

B.D., A.C., P.R. were responsible for the concept and design of the study. B.D., A.C., P.R., S.S., and T.K. were responsible for the acquisition, analysis, and interpretation of data. B.D. and A.C. were responsible for drafting the article. B.D., A.C., P.R., T.K., and S.S. were responsible for revising the article critically for intellectual content. All authors gave final approval of the final version to be published.

Disclosure Statement

No competing financial interests exist.

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