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Air and Water Processes Do Not Produce the Same High-Quality Pasteurization of Donor Human Milk

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Abstract

Background: Holder pasteurization is the most commonly used technique in milk banks worldwide, but higher temperatures and longer pasteurization time have been associated with damage to the immune components of human milk.
Research aim: This study aimed to assess the detailed pattern of pasteurization temperature using two water pasteurizers (WP1 and WP2) and one air pasteurizer (AP).
Methods: The milk temperature during each phase of the pasteurization cycle was recorded using 6 to 9 probes, depending on the number of bottles, in the pasteurizers. We used 90 to 200 ml bottles to assess the effect of volume on milk temperature.
Results: The time to heat the milk from room temperature to 58°C was 12.4, 12.9, and 64.5 min, respectively, for WP1, WP2, and the AP ($p < .0001$). The duration of the plateau was 35.5, 35.2, and 45.8 min ($p < .0001$). The duration of exposure to a temperature above 58°C was 49.6, 40.7, and 76.2 min ($p < .0001$). The total duration of a full cycle was 79, 66, and 182 min ($p < .0001$). The duration of exposure above 58°C for the different volumes of milk treated showed no difference when using WP1 but was significantly longer in small volumes when using WP2.
Conclusion: Human milk treated using the air pasteurizer in our study was exposed to higher temperatures and for longer periods of time than the water pasteurizers we employed. Regular qualification of pasteurizers is requested when evaluating the effect of pasteurization on milk components and for routine treatment of human milk in milk banks.

Keywords

breastfeeding, human milk, infant nutrition, milk bank, milk banking, nutrition policy

The reference technique used to pasteurize human milk (HM) is the holder method, which uses a low temperature and a long period of pasteurization (LTLT) to heat the milk at 62.5°C for 30 min. This reference technique was published by Nicolas Appert (1831) and patented in 1865 by Louis Pasteur for pasteurizing wine (Institut Pasteur, 2016). The dairy industry used holder pasteurization first to protect bovine milk from bacteria. Milk was then heated in trays called holders, from which the holder method got its name. Most of the HM banks (HMBs) worldwide now use this technique, as it is recommended by most international guidelines (PATH, 2013). It currently offers the best compromise among safety, namely, destruction of microorganisms; preservation of the quality components in HM; and feasibility.

Pasteurization preserves most of the macronutrients, oligosaccharides, vitamins, and growth factors and some cytokines (ESPGHAN Committee on Nutrition et al., 2013; Peila et al., 2016; Unger, Gibbins, Zupancic, & O'Connor, 2014) but destroys other HM components, such as lactoferrin and immunoglobulins. In 1978, Evans, Ryley, Neale, Dodge, and

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Lewarne showed that the loss of immunoglobulin G (IgG), immunoglobulin A (IgA), lysozyme, and lactoferrin was more likely to occur when the temperature increased above 60°C, which was later confirmed by Czank, Prime, Hartmann, Simmer, and Hartmann (2009). These authors showed that the loss of immunological components was stable from 40°C to 57°C but that it increased dramatically above 57°C. Therefore, those who use this technology must ascertain that the pasteurizers are able to expose HM to the right temperature (62.5°C) for the right time (30 min), with a rapid increase and quick decrease in the milk temperature and accurate control of the plateau duration. The effect of LTLT on HM components has been extensively analyzed, but none of these studies have produced data about the criteria for the devices that have been used, which could explain some discrepancies (Peila et al., 2016).

Different types of pasteurizers are presently available on the market and pasteurization can be processed in water, which is the most common method, or in air. Air pasteurizers are less expensive and have been bought by some HMBs. However, to our knowledge, no data are available on the effectiveness of this method for treating HM. Browsing the leaflets of the major pasteurizers' manufacturers, none show the distribution of the temperature in the bottles, and no clear evaluation of the performance of these pasteurizers is reported. They contain only an imprecise curve recorded in one bottle and few details on time exposure of the milk. Our aim was to compare the pasteurization temperatures obtained using three different devices—two using water and one using air.

Methods

Design

A three-group longitudinal observational design was used to meet the study aim: We recorded temperature of pasteurization cycle in three different types of pasteurizers to compare them. The three devices used were the Climats® air pasteurizer (AP), which pasteurizes, cools, and freezes HM (Climats, Saint Médard d'Eyrans, France), and two HSC® water pasteurizers, the PAS 10000 (WP1) and the PAS 10002 (WP2), which pasteurize and cool HM (HSC, Décines, France). The two water devices were similar, except that WP2 is the more recent version of the HSC device and provides more precise temperature regulation than WP1. Furthermore, WP2 can cool the milk until it reaches 4°C instead of the ambient tap water temperature offered by WP1. These pasteurizers are the three models used in our milk bank and in the majority of French HMBs. Milk was placed in single-use Beldico® polypropylene bottles (Beldico SA, Aye, Belgium). No ethical consent was needed for this study, according to French law.

Settings

This study was performed at the regional HMB at the Croix-Rousse University Hospital in Lyon, France, in May 2015.

Key Messages

- Despite being the worldwide reference method, holder pasteurization alters the immune components of human milk.
- The alteration of milk components depends on the duration and level of the temperature used.
- We found that the duration and level of the temperature exposure differed depending on the type of pasteurizer.
- Pasteurization temperature was more homogeneous in water than in air.
- Regular qualification of pasteurizers is needed to optimize pasteurization and milk quality.

Our HMB is regional and represents the second biggest milk bank in France, processing 9,000 L of HM per year for a region of 70,000 km^2.

Sample

To monitor the temperature during the pasteurizer cycle, recording probes certified by the French Accreditation Committee were introduced into different bottles and evenly distributed in the three devices (see Figure 1). Because the composition in water of bovine milk is similar to HM, the difference in transmission of heat is negligible. Therefore, we decided to avoid wasting HM and filled the probes' bottles with bovine milk. The other bottles were filled with HM pasteurized as usual, according to the current French recommendations (Afssaps, 2008). The bottles were filled with 90 to 200 ml of milk for the WP1 and WP2 methods and 200 ml for the AP method, as this is routine practice in our HMB.

Measurements

Six probes were used for the 48 bottles in WP1 and 9 probes were used for the 63 bottles in WP2 and the 90 bottles in the AP. The distribution of the probes in the pasteurizers is shown in Figure 1. The temperature was recorded every 2 s for each cycle of pasteurization, and 3 sets of pasteurization cycles were recorded for the study in the following order: WP1, WP2, and AP.

Data Collection

We defined 10 quality control parameters that needed to be assessed (see Figure 2). These included how long the milk was exposed to a temperature of ≥ 58°C, which is crucial, as significant changes in HM components occur above this temperature (Czank et al., 2009). This exposure should be as short as possible. Indeed, because of inertia, the time for the milk to increase from 58°C to 62.5°C is the most difficult part of heating for the pasteurizers, and as the plateau

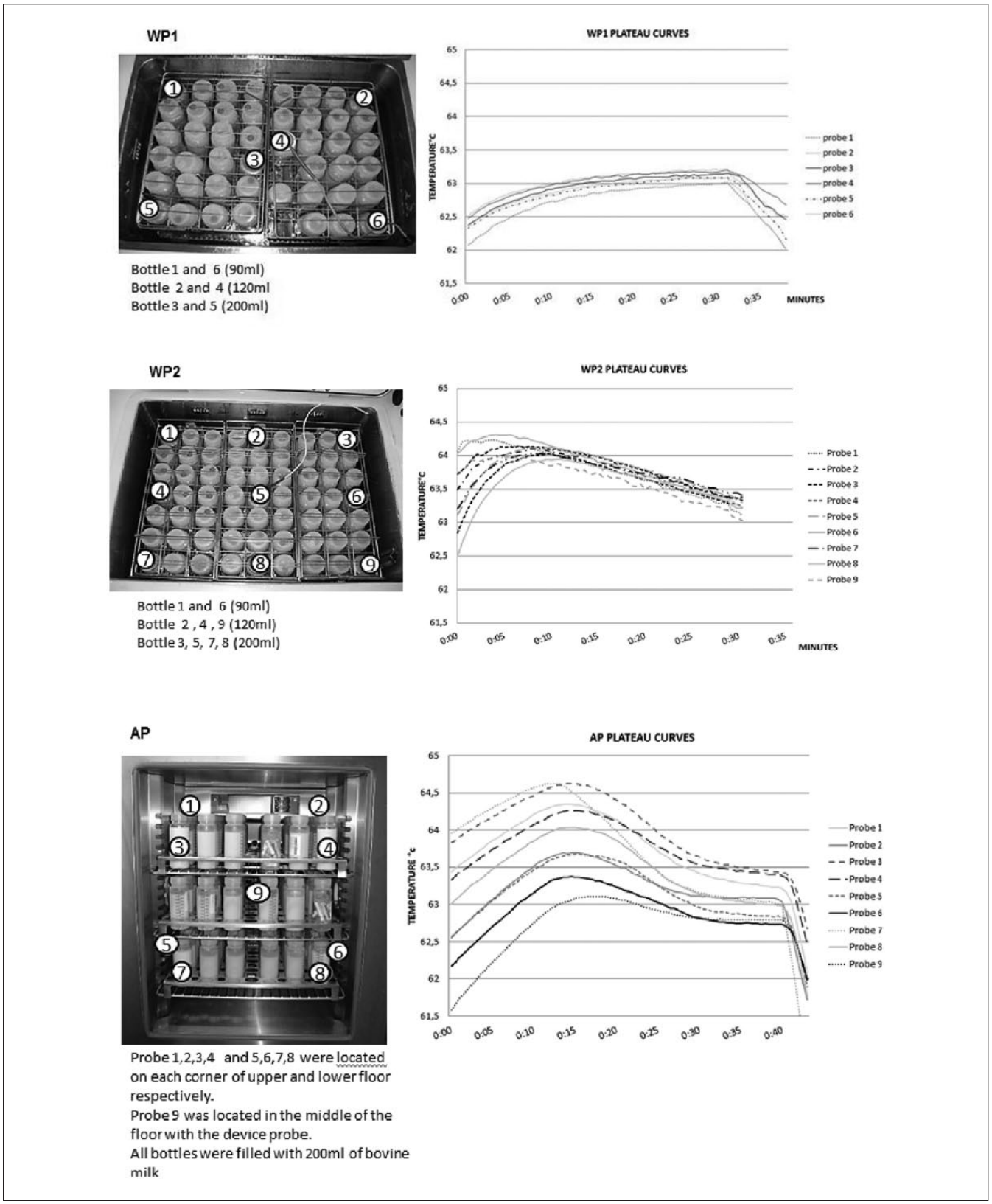

Figure 1. Results of the temperature assessments of each probe during a pasteurization cycle in three different pasteurizers: water pasteurizer 1 (WP1), water pasteurizer 2 (WP2), and air pasteurizer (AP).

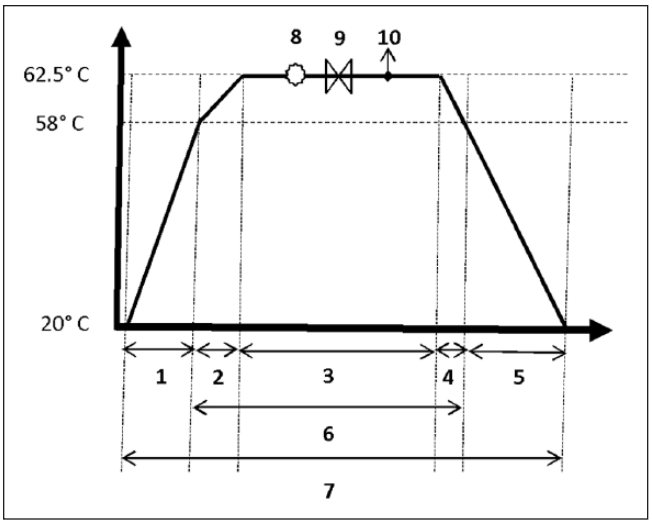

Figure 2. Schematic representation of a pasteurization cycle, with the assessment of 10 parameters: (1) time from room temperature 20°C to 58°C, (2) time from 58°C to 62.5°C, (3) duration of plateau, (4) time from 62.5°C to 58°C, (5) time from 58°C to 20°C, (6) duration of exposure over 58°C, (7) total duration of the pasteurization cycle, (8) mean temperature, (9) time between the first and the last probe reaching 62.5°C, and (10) highest temperature during plateau.

during holder pasteurization lasts 30 min, it leaves 20 min for this part of the heating process and for the decrease from 62.5°C to 58°C. Currently, there are very few pasteurizers able to heat the milk over 58°C for less than 50 min. Therefore, within the limits of the technology, a maximum duration of 50 min is feasible and limits the damage caused to the milk components. The temperature during plateau should stay as close as possible to 62.5°C, which represents the lower limit, as bactericidal efficacy has been shown at this level. The upper limit should not be too far from 62.5°C but should be feasible with the current technology. Therefore, a range of 62.5°C to 64°C is recommended. The duration of the plateau should be between 30 and 35 min, as it is close enough to the duration validated for LTLT, and the plateau should start when all the probes achieve the right temperature of 62.5°C. The dairy industry maintains that milk must be refrigerated within an hour, at less than 6°C, to avoid the proliferation of any bacteria that have not been destroyed by the pasteurization process (Décret n°55-771, 1955). This rapid cooling also creates a thermal shock that minimizes the growth of spores without altering the immunological components (Royal College of Paediatrics and Child Health, 2003). To evaluate the homogeneity of the pasteurizers, we also calculated the difference in time between the first and the last probe reaching 62.5°C (see Figure 2). To evaluate the effect of the volume on heating in the WPs, we assessed the durations of the plateau and exposure over 58°C for the three different volumes in the bottles: 90, 120, and 200 ml.

Data Analysis

The data of each recording probe were gathered in an Excel spreadsheet, version 2010 (Microsoft Corporation, Redmond, WA). The time for each session during the cycle was calculated when each probe reached the target temperature. The mean temperature for each pasteurizer for the 10 different endpoints is the average temperature for all probes and for the three cycles with its 95% confidence interval estimated using the linear mixed-effects model, taking into account the correlation of measurements within probes and within cycles. A p value below .05 was considered statistically significant. The analyses were performed using R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The temperature of the milk during every phase of the pasteurization cycle using each pasteurizer is shown in Table 1. There were significant differences between the three pasteurizers in all of the quality control parameters, and these differences were mostly between the AP and the two WPs (see Table 1). Intercycle and interprobe variabilities are shown in Supplementary Table 1. It took 5 times longer to heat the milk to 58°C in the AP than with WP1 or WP2 ($p < .001$), and the plateau duration was 30% longer in the AP than WP1 and WP2 ($p < .001$). The exposure time over 58°C in the AP was 1.5 and 1.9 times longer than in WP1 and WP2 ($p < .001$), respectively (see Table 1). The time between the first and the last probe reaching 62.5°C was 5.8 times longer in the AP than in WP1 or WP2 (see Table 1). The homogeneity of the temperature between each probe in the different bottles during each pasteurizer's cycle is shown in Figure 1. These curves also show that the plateau temperature remained between 62.5°C and 64.5°C for all probes when using WP1 and WP2, but in only 7 of 9 probes when using the AP. Finally, the comparison between the mean temperature curves in each pasteurizer is shown in Figure 3. The duration of a full cycle with the AP was 2.3-fold and 2.8-fold longer than with WP1 and WP2, respectively. With regard to the volume of the bottles used in the WPs, a significant association was observed between volume and plateau duration with WP1, with a longer duration in the larger bottles, but there was no statistical difference for the duration of exposure over 58°C. There was a statistical difference in the plateau duration with WP2 when different volumes were used, and in exposure to temperatures higher than 58°C (see Table 2), but the difference was less than 2 min.

Discussion

Our study found significant differences in the milk exposure to temperature depending on whether we used a water- or air-based pasteurizer. The pasteurization temperature was much more precisely and homogeneously applied to the

Table 1 Mean Durations [and 95% Confidence Intervals] of 10 Quality Control Time Parameters of Pasteurization Cycles With Three Different Pasteurizers

	WP1	WP2	AP	p_1 [a]	p_2 [b]
1. Time from room temperature to 58°C	12.4 [10.7, 14.2][c]	12.9 [11.5, 14.4]	64.5 [63.1, 65.9]	< .001	.657
2. Time from 58°C to 62.5°C	12.4 [11.6, 13.2]	4.9 [4.3, 5.6]	24.3 [23.6, 24.9]	< .001	< .001
3. Duration of plateau	35.5 [33.0, 38.1]	35.2 [33.1, 37.3]	45.8 [43.7, 47.9]	< .001	.826
4. Time from 62.5°C to 58°C	1.6 [1.3, 2.0]	0.6 [0.4, 0.9]	6.1 [5.8, 6.3]	< .001	< .001
5. Cooling time down to 20°C	17.1 [16.1, 18.2]	12.3 [11.4, 13.1]	41.2 [40.3, 42.0]	< .001	< .001
6. Time exposure over critical temperature of 58°C	49.6 [47.4, 51.8]	40.7 [38.8, 42.6]	76.2 [74.3, 78.1]	< .001	< .001
7. Duration of full cycle	79.2 [77.4, 81.0]	66.0 [64.5, 67.4]	182 [180, 183]	< .001	< .001
8. Mean temperature of the probe during plateau (°C)	63.0 [62.9, 63.1]	63.6 [62.5, 63.7]	63.3 [63.3, 63.4]	< .001	< .001
9. Time between the first and the last probe for reaching 62.5°C	3.7 [0.6, 6.8]	5.0 [1.9, 8.1]	21.7 [18.6, 24.8]	.001	.546
10. Highest temperature (°C)	63.2 [63.0, 63.3]	64.0 [63.9, 64.2]	63.9 [63.8, 64.1]	< .001	< .001

Note. Two water pasteurizers (WP1 and WP2) and one air pasteurizer (AP) were used. Time is given in minutes.
[a]p_1 = p value between all three pasteurizers. [b]p_2 = p value between WP1 and WP2. [c]95% confidence intervals are estimated using a linear mixed-effects model.

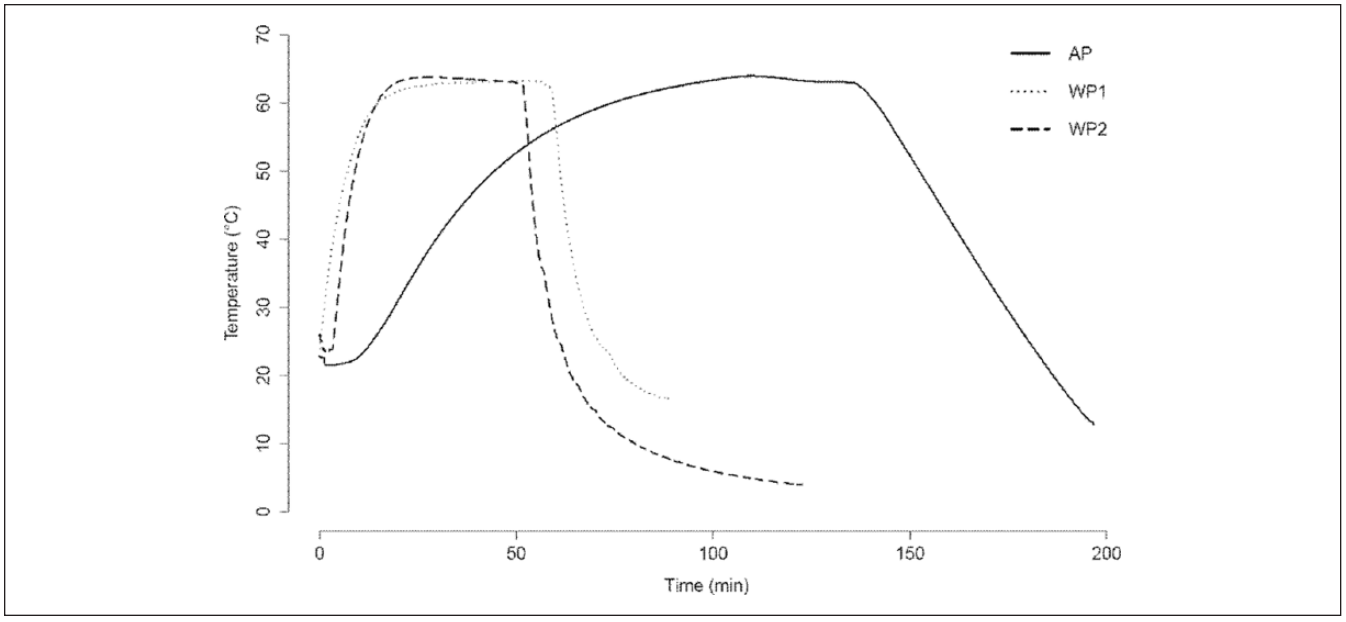

Figure 3. Comparison of the temperature curves over time for a pasteurization cycle in each pasteurizer: water pasteurizer 1 (WP1), water pasteurizer 2 (WP2), and air pasteurizer (AP).

bottles of milk with the two WPs than with the AP. It has previously been shown that the duration of exposure to high temperatures and the level of temperature have a significant effect on the immunologic components of HM (Czank et al., 2009; Evans et al., 1978). In 1978, Evans and colleagues (1978) showed that the loss of IgG, IgA, lactoferrin, and lysozyme started at a temperature of 60°C, but this loss was very important at 65°C. In a more recent study, Czank et al. (2009) described the precise loss of immune components of HM depending on the level of temperature and the duration of heating. The authors showed that the loss was similar between 40°C and 57°C and became more important as soon as the temperature reached 58°C and increased above that level. We observed that the average time that elapsed between 58°C and 62.5°C was 2 to 4 times longer with the AP than with WP2 and WP1, respectively. The improved temperature regulation in WP2 halved the exposure to these temperatures, avoiding the inertia observed with WP1. Moreover, Czank et al. (2009) showed that at 62.5°C, there was a decrease of 1.6%, 1.7%, and 2.4% per minute, respectively, of the IgA, lysozyme, and lactoferrin concentrations in HM. Therefore, we could estimate that the preservation of IgA, lysozyme, and lactoferrin would be 62%, 60%, and 48%, respectively, after 30 min of exposure to 62.5°C. Since the

Table 2 Mean Durations of the Plateau and Exposure Time Over 58°C in Two Different Water Pasteurizers (WP1 and WP2), According to Bottle Volume

	90 ml	120 ml	200 ml	p[a]	Intercycle variability
WP1					
Plateau duration	33.8 [31.8, 35.9][b]	36.2 [34.1, 38.2]	36.6 [34.6, 38.6]	.03	1.188[b]
Time exposure above 58°C	49.4 [47.5, 51.4]	50.1 [48.2, 52.0]	49.2 [47.3, 51.2]	.29	1.402
WP2					
Plateau duration	36.0 [34.7, 37.2]	36.3 [35.2, 37.3]	33.9 [33.0, 34.9]	.001	0.484
Time exposure above 58°C	41.4 [40.3, 42.4]	41.6 [40.7, 42.5]	39.8 [38.9, 40.6]	.004	0.352

Note. Time is given in minutes.
[a]p value between all three volumes. [b]95% confidence intervals and intercycle variability (standard deviation) are estimated using a linear mixed-effects model.

plateau duration differed by about 10 min between the AP and WP1 or WP2, one could expect a 9% to 10% higher loss with the AP just for the plateau's duration. But if we consider the time of exposure over 58°C, it was much longer with the AP, at 26 min longer than WP1 and 36 min longer than WP2. Such differences in temperature exposure could explain the discrepancies observed in studies that focused on the loss of immune factors in HM during pasteurization (Peila et al., 2016). With regard to the different volumes of milk in the bottles pasteurized at the same time, the difference in the duration of the plateau when using WP1 was about 2.5 min between a volume of 90 ml and a volume of 200 ml. However, there was no difference in the duration of exposure to temperatures higher than 58°C. In WP2, the difference was also statistically significant, but such a short difference in heat exposure, of less than 2 min, is unlikely to have had a significant effect on the loss of immune components. The good homogeneity of exposure to temperature produced by the WPs seems to have allowed reliable pasteurization of different volumes in the same pasteurization cycle.

Since air is a poor heat conductor, the bottles of milk in the AP did not reach the target temperature at the same time, which means that some bottles were heated for a longer time than others during the same pasteurization cycle. The pasteurization was extremely heterogeneous with the AP, with a difference in the plateau duration of up to 30 min from one probe to another. When the temperature applied to HM is homogeneous in the different bottles, the adjustment of temperature settings is uniform in all bottles. Thus, this can be checked once a year by qualified probes, certified, external to the pasteurizer, and placed in bottles distributed in several places in the pasteurizer. Such probes can accurately monitor the temperature during the whole cycle and in several places in the pasteurizer and determine whether the temperature value measured by the pasteurizer's probe is correct. When temperature is controlled with qualified probes once a year, using one single probe in routine monitoring is sufficient. That would not be the case using the AP.

The amount of time that the milk was exposed to harmful temperatures of higher than 58°C was 9 min less in WP2 than WP1, but the temperature applied to the HM using WP2 still reached 64°C. Further improvements to the technology could help to lower the temperature closer to 62.5°C, to optimize the pasteurization cycle and preserve the immune components. Moreover, as the full cycle of heating, plateau, and cooling was twice as long with the AP than the WPs, this extra time may hamper the functioning of HMBs that need to carry out a number of pasteurization cycles on the same day.

Limitations

A limitation of our study was that the volumes were identical in the bottles in the AP, at 200 ml, but were 90 to 200 ml in the bottles in WP1 and WP2. However, the heating time in the AP was different among the bottles, as there was a 21.5-min difference between the first and the last probes reaching the same temperature. The heating times were very similar between the different bottles processed by WP1 and WP2 at 3.19 to 4.43 min, which means that the pasteurization time of different volumes was not very different. It seems that different volumes of HM can be efficiently pasteurized at the same time when using WPs. Although it was not evaluated in the present study, it is likely that the longer time that it took for the AP to process the milk would have increased the loss of immune components for smaller volumes of HM. This is relevant for the daily practice of HMBs, which often have to treat variable amounts of milk.

Another limitation of our study is that we focused on exposure to temperature and the time that the milk was exposed to heat, and we did not evaluate the precise effect that the different pasteurizers had on the components in the milk. Data from the literature show that differences, such as those observed in our study, are likely to significantly alter the quality of HM (Czank et al., 2009; Evans et al., 1978). We did not evaluate the effect of the differences between the pasteurizers on the microbiological efficacy of the pasteurization process. Holder pasteurization has previously been shown to destroy most bacteria and viruses in HM. Czank et al. (2009) showed that 58°C was the right temperature to eliminate the majority of pathogens, such as *Escherichia coli, Staphylococcus epidermidis, Enterobacter cloacae*, and *Staphylococcus aureus*, within 30 min. With regard to

Table 3 Proposed Criteria for Qualification of Human Milk Pasteurizers

- Qualification repeated once a year and after major intervention
- Performed on three pasteurization cycles in real conditions (loaded)
- Measurement by independent qualified temperature probes (different from those supplied with pasteurizers)
- One probe for 8 to 10 bottles and distributed regularly in the bath
- Temperature plateau of 62.5°C to 64°C for each probe
- Duration of the plateau (time counted when all probes are at 62.5°C) between 30 and 35 min (safety)
- Exposition time over 58°C < 50 min for each probe (quality)
- Exposition time from 62.5°C to 6°C ≤ 1 hr

viruses, Welsh, Arsenakis, Coelen, and May (1979) demonstrated that reductions in the herpes simplex virus type 1, Coxsackies virus, and Semlicki Forest virus were the same between 56°C and 62.5°C for 30 min, but the cytomegalovirus persisted. It could be interesting to test different heating temperatures that would eliminate the pathogens but preserve the maximum value of the immune components of HM. Other thermal or nonthermal techniques have been proposed that seem to preserve more HM components, such as high-pressure processing, ultraviolet-C, and high-temperature short-time pasteurization, but they are difficult to use to treat large volumes of HM in an HMB (Chantry et al., 2011; Christen, Lai, Hartmann, Hartmann, & Geddes, 2013; Peila et al., 2017; Sousa, Delgadillo, & Saraiva, 2016). These new techniques should be compared with reference holder pasteurization using qualified pasteurizers.

Although holder pasteurization remains the mostly widely used technique by HMBs, there is no consensus about the quality control of pasteurizers to ensure the smooth operation of the different devices that are available. There are cruel deficiencies from the commercial pasteurizers' companies to produce quality control processes. The lack of standardization of the process explains the important discrepancies that can be observed in the results of the holder method in the literature. However, standards exist: as the European standard NF EN 60068-3 for the characterization of climatic chambers. These types of chambers approach pasteurizers, and elements of their characterization are well summarized in Blanquart and Crétinon's (2013) article. Using this example, the studies of the dairy industries, and the results of recent studies—including ours, simple quality control and safety criteria could be proposed in order to characterize the pasteurizers used by HMBs (see Table 3).

Conclusion

Our study found that different types of air- and water-based pasteurizers did not reproduce the same pattern of holder pasteurization and that some of the temperature patterns could significantly alter the properties of HM. As holder pasteurization is the most widely used technique by HMBs, it should be controlled and optimized. The efficacy of pasteurizers should be qualified by the manufacturers and regularly confirmed by the users.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplementary Material

Supplementary material for this article is available online.

References

Afssaps. (2008). Règles de bonnes pratiques de collecte, de préparation, de qualification, de traitement, de conservation, de distribution et de délivrance sur prescription médicale du lait humain par les lactariums; Décision du 3 décembre JO du 5 janvier 2008 [The good practice rules for the collection, preparation, qualification, treatment, storage, distribution and dispensing on medical prescription of human milk by the milk banks]. Retrieved from http://association-des-lactariums-de-france.fr/wp-content/uploads/lactarium_guide_bonnes_pratiques_5_janvier_2008_traduction_anglais.pdf

Appert, N. (1831). *Le livre de tous les ménages ou l'art de conserver pendant plusieurs années toutes les substances animales et végétales* [The book of all households or the art of preserving for several years all the animal and vegetable substances] (4th ed.). Paris, France: Barrois l'aine.

Blanquart, B., & Crétinon, B. (2013). Maîtrise des enceintes thermostatiques dans les laboratoires: le début des difficultés? [Control of thermostatic enclosures in laboratories: The beginning of difficulties?]. Retrieved from https://doi.org/10.1051/metrology/201315008

Chantry, C. J., Wiedeman, J., Buehring, G., Peerson, J. M., Hayfron, K., K'Aluoch, O., . . . Abrams, B. (2011). Effect of flash-heat treatment on antimicrobial activity of breastmilk. *Breastfeeding Medicine*, *6*(3), 111-116. Retrieved from https://doi.org/10.1089/bfm.2010.0078

Christen, L., Lai, C. T., Hartmann, B., Hartmann, P. E., & Geddes, D. T. (2013). Ultraviolet-C irradiation: A novel pasteurization method for donor human milk. *PloS One*, *8*(6), e68120. Retrieved from https://doi.org/10.1371/journal.pone.0068120

Czank, C., Prime, D. K., Hartmann, B., Simmer, K., & Hartmann, P. E. (2009). Retention of the immunological proteins of

pasteurized human milk in relation to pasteurizer design and practice. *Pediatric Research*, *66*(4), 374-379. Retrieved from https://doi.org/10.1203/PDR.0b013e3181b4554a

Décret n°55-771 du 21 mai 1955 relatif aux laits destinés à la consommation humaine [Decree no. 55-771 of May 21, 1955, relating to milks intended for human consumption].

ESPGHAN Committee on Nutrition, Arslanoglu, S., Corpeleijn, W., Moro, G., Braegger, C., Campoy, C., . . . van Goudoever, J. (2013). Donor human milk for preterm infants: Current evidence and research directions. *Journal of Pediatric Gastroenterology and Nutrition*, *57*(4), 535-542. Retrieved from https://doi.org/10.1097/MPG.0b013e3182a3af0a

Evans, T. J., Ryley, H. C., Neale, L. M., Dodge, J. A., & Lewarne, V. M. (1978). Effect of storage and heat on antimicrobial proteins in human milk. *Archives of Disease in Childhood*, *53*(3), 239-241.

Institut Pasteur. (2016). *Biographie de Louis Pasteur*. Retrieved from http://www.pasteur.fr/fr/institut-pasteur/l-histoire/louis-pasteur/biographie-louis-pasteur

PATH. (2013). *Strengthening human milk banking: A global implementation framework* (Version 1.1). Seattle, WA: Author.

Peila, C., Emmerik, N. E., Giribaldi, M., Stahl, B., Ruitenberg, J. E., van Elburg, R. M., . . . Cavallarin, L. (2017). Human milk processing: A systematic review of innovative techniques to ensure the safety and quality of donor milk. *Journal of Pediatric Gastroenterology and Nutrition*, *64*(3), 353-361. Retrieved from https://doi.org/10.1097/MPG.0000000000001435

Peila, C., Moro, G. E., Bertino, E., Cavallarin, L., Giribaldi, M., Giuliani, F., . . . Coscia, A. (2016). The effect of holder pasteurization on nutrients and biologically-active components in donor human milk: A review. *Nutrients*, *8*(8). Retrieved from https://doi.org/10.3390/nu8080477

Royal College of Paediatrics and Child Health. (2003). *Guidelines for the establishment and operation of human milk banks in the UK* (3rd ed.). London, UK: Author.

Sousa, S. G., Delgadillo, I., & Saraiva, J. A. (2016). Human milk composition and preservation: Evaluation of high-pressure processing as a nonthermal pasteurization technology. *Critical Reviews in Food Science and Nutrition*, *56*(6), 1043-1060. Retrieved from https://doi.org/10.1080/10408398.2012.753402

Unger, S., Gibbins, S., Zupancic, J., & O'Connor, D. L. (2014). DoMINO: Donor milk for improved neurodevelopmental outcomes. *BMC Pediatrics*, *14*, 123. Retrieved from https://doi.org/10.1186/1471-2431-14-123

Welsh, J. K., Arsenakis, M., Coelen, R. J., & May, J. T. (1979). Effect of antiviral lipids, heat, and freezing on the activity of viruses in human milk. *The Journal of Infectious Diseases*, *140*(3), 322-328.