Effect of Alternative Pasteurization Techniques on Human Milk's Bioactive Proteins

Effect of alternative pasteurization techniques on human milk's bioactive proteins

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Authors’ contributions

A.A., L.Ca., M.G., V.Z., and L.Co. conceptualized the study.

A.A., L.Ca., M.G., and L.Co. discussed and agreed on study methodology.

L.Ca., M.G. and S.A. performed data analysis.

S.M. and F.V. were in charge of data curation.

A.A., L.Ca. and M.G. wrote the original draft of the paper.

A.A., L.Ca., S.M., M.G., F.V., S.A., V.Z., and L.Co all contributed to paper’s review and editing.

Each author has approved the submitted version and agrees to be personally accountable for the author’s own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

Abstract

Objectives

Human milk (HM) feeding leads to improved outcome for preterm infants. When mother's milk is unavailable, pasteurized donor HM (DHM) is the recommended alternative over formula. The Holder pasteurization (HoP) method is universally performed in HM banks; however, it is known to impair several functional HM components.

The aim of this study was to compare the efficacy of HoP vs. two innovative processing methods (High-Temperature-Short-Time [HTST] pasteurization and High-Pressure-Processing [HPP]) in preserving some bioactive HM protein components.

Methods

HM samples from donors of the Bologna HM bank were collected and divided into 4 subsamples: one was kept raw, and each of the others was processed using a different technique (HoP, HTST, and HPP). Total protein (TPC), secretory immunoglobulin A (sIgA), and lactoferrin content were compared.

Results

Both HM lactoferrin and sIgA content were negatively affected, but to a different extent, by each method: sIgA was better preserved by HTST (-23.3%) with only HPP leading to a significant reduction (-35.8%); lactoferrin content was strongly reduced by HoP (-87.5%) and HTST (-83.5%), and preserved by HPP (-24.7%). Variations in protein profile were seen for all processing methods, being more relevant for HoP, followed by HTST and, finally, by HPP. All the three methods reduced starting HM microbial counts to undetectable level.

Conclusions

Both HTST and HPP better preserved the original HM protein profile, compared to HoP. However, they affected differently some bioactive HM components involved in immune response and antibacterial activity.

Keywords

Donor human milk, human milk pasteurization, protein profile, lactoferrin, secretory IgAs

What is known

- Donor human milk (DHM) represents the best alternative to own mother's milk for preterm infants
- To comply with microbiological safety standards, DHM is usually pasteurized in HM banks using the Holder (HoP) method, which is known to impair several DHM bioactive properties
- Pasteurization methods alternative to HoP such as high-temperature-short-time (HTST) and high-pressure-processing (HPP) are currently under investigation

What is new

- Both HTST and HPP comply with microbiological safety standards
- Secretory immunoglobulin A are better preserved by HTST; lactoferrin content is strongly reduced by both HoP and HTST, and preserved by HPP.
- The highest variations in protein profile are seen after HoP.

45 *Introduction*

46 Human milk (HM) represents the optimal feeding for preterm infants, especially for those born with
47 a very low (<1500 g) birth weight (1). Mother's own milk is uniquely tailored for each newborn,
48 both in its nutritional composition and in the non-nutritive bioactive factors that promote survival
49 and healthy development (2). When mother's own milk is unavailable or insufficient, the use of
50 donor HM (DHM) is recommended (3): recent studies have shown that DHM-feeding is associated
51 with a reduction of neonatal morbidities including necrotizing enterocolitis (4,5). Furthermore, it
52 has been documented that the availability of DHM has a paradoxical beneficial effect in increasing
53 the rates of breastfeeding among mothers who deliver prematurely (4,6). To ensure its quality and
54 safety, DHM must be provided by a HM bank (HMB) (3): specific recommendations for the
55 preparation, pasteurization, and distribution of DHM exist in many countries worldwide (7–12) . As
56 for the process of pasteurization, the Holder pasteurization (HoP) method is universally
57 recommended by all HMBs, because, at present, it is the only method for which validated devices
58 are commercially available, and for which an extensive amount of evidence on safety and efficacy
59 exists (8,13–15). However, it is well known that HoP, which is a thermal process performed on
60 bottled DHM at 62.5°C for 30 minutes, followed by fast cooling to a temperature below 4°C,
61 impairs several functional HM components, including immunoglobulins (Ig), lactoferrin, lipases, as
62 well as other enzymatic activities, some cytokines and some vitamins (8,13–15). For this reason,
63 further research is currently being aimed at testing pasteurization methods alternative to HoP, which
64 would be capable of retaining the largest variety of HM bioactive properties to the highest extent,
65 without affecting DHM microbiological safety (8,13–16).

66 Several alternative pasteurization methods are being investigated in recent years: these include
67 thermal processes, such as High-Temperature-Short-Time pasteurization (HTST), and non-thermal
68 methods, such as High-Pressure Processing (HPP) and ultraviolet irradiation, as well mixed
69 techniques, such as (thermo-)ultrasonic processing (8,13,15–17). HTST and HPP are considered as
70 the most promising alternatives to HoP for DHM (16,17), being the most widely studied at present.

The main limitation to their applicability as routine pasteurization techniques in HMBs is the lack of specific instrumentation, validated in relevant HMB environment. This technical gap is being progressively filled, since specific devices to be operated with small milk volumes have been recently developed for the use in HMBs (18,19).

The aim of the present study is to contribute to the innovation in the field by directly comparing the efficacy of the two most promising techniques, HTST and HPP, to standard HoP. To this aim, prototyped pasteurization equipment and commercial devices are used. Changes in the protein profile of DHM, as well as specific HM bioactive components (lactoferrin and secretory IgA content [sIgA]), are addressed.

Methods

- *Ethics*

The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi Hospital, Bologna, Italy (study # 165/2015/U/Tess). HM samples were collected from donors of the Bologna HMB, after signing an informed written consent.

- *Collection of HM samples*

HM samples were collected, approximately 3 months after delivery, from five HM donors following the requirements of the Bologna HMB. Mothers were asked to express milk by a breast pump after carefully washing their hands and breast. HM was collected using sterile, single-use breast pump kits into sterile polypropylene bottles. HM samples were stored at -20°C at the HMB until processed, and then handled following the routine protocol used for DHM (11): they were removed from the freezer several hours before the analyses and processed only when completely thawed.

- *HM processing*

HM pools (400 ml each) were obtained from individual donors by collecting milk during few consecutive days. Each milk pool was divided into four sub-samples (100 ml each): one sample was not pasteurized, one sample was pasteurized by HoP, one by HTST, and the last by HPP. Each sub-sample was analysed for selected indicators of protein and microbial quality. HoP was performed at the Bologna HMB, following the standard pasteurization procedure for DHM which is used in our neonatal intensive care unit. Specifically, HoP was performed by a standard HM Holder pasteurizer (S90 TES, Medicare Colgate LTD, Cullompton, UK). DHM was pasteurized using a temperature of 62.5°C for 30 minutes (tolerance ±0.5°C), and then cooled to 4°C in 60 minutes (tolerance ±0.5°C). HPP was performed at HPP Italia, Traversetolo, Italy, using an industrial AV-30 device produced by Avure Technologies, Inc. (Middletown, OH, United States); HPP was performed by applying a 6000 bar pressure for 3 minutes to bottled HM. HTST pasteurization was performed as previously described (19), using a patented proprietary device (Patent number: EP 15176792.8-1358), which is a bench-top device consisting in a system of tubular heat exchangers for heating as well as for cooling. The temperature was monitored along the pasteurization steps by specific digital probes (tolerance ±0.5°C).

- *HM analyses*

Pasteurized (HoP, HPP and HTST), and unpasteurized HM were assayed for total protein, sIgAs and lactoferrin content, and for protein profile. sIgAs were measured on 1:10,000 diluted samples, using an ELISA kit (Biovendor, Brno, Czech Republic) and following the manufacturer's instructions, in triplicate. Lactoferrin quantity was determined in triplicate on 1:20,000 diluted samples (1:40,000 for unpasteurized HM), using an ELISA kit (Biovendor) and following the manufacturer's instructions. Total protein content (TPC) was determined on samples skimmed by centrifugation at 2,000g at 4 °C for 30 min, using 2DQuant kit (GE Healthcare Italia, Milan, Italy), in duplicate, following the manufacturer’s instructions. The protein profile (in non-reducing

conditions, 5 µg of proteins) was visualized by monodimensional electrophoresis on a 10-well 12% Nu-PAGE® precast gel (Thermo Fisher Scientific, Waltham, MA, United States) with MES (Thermo Fisher Scientific) as running buffer, on a Novex Mini-cell (Thermo Fisher Scientific) at 200 V. The gels were stained with Blue Coomassie Colloidal stain, following the protocol already described in a previous study (20).

Microbiological safety analyses were performed following the Italian guidelines for HMBs (11), on both unpasteurized and pasteurized HM samples, to verify the compliance of each pasteurization technique to the HMB requirements. Each HM sample was assessed for total bacterial load (Plate Count Agar [PCA] - Kima Meus, Piove di Sacco, Italy), presence of Enterobacteriaceae (Herellea Agar - Kima Meus) and *Staphylococcus aureus* (Mannitol salt agar - Kima Meus). Bacterial counts were reported as Colony Forming Units (CFU)/mL. In order to comply with the standards required by HMB guidelines (11), in raw HM total viable bacteria count must be $<10^5$ CFU/ml, and both Enterobacteriaceae and *S. aureus* $< 10^4$ CFU/ml; after pasteurization, no bacteria should be detected.

- *Statistical analysis*

TPC, lactoferrin, and secretory IgA contents were reported as median values and interquartile ranges. In order to evaluate difference among unpasteurized HM and the three different pasteurization methods, one-way ANOVA with Tukey post-hoc comparison was used for TPC and sIgA, and Kruskal-Wallis with Steel-Dwass post-hoc comparison for lactoferrin. A p-value <.05 was considered as statistically significant.

Results

- *Protein fraction analysis*

Results of protein fraction analyses are summarized in Table 1. TPC of defatted HM was quantified before and after each processing method. TPC of the five analysed HM samples before

148 pasteurization varied between 5 and 12.1 g/L (median: 7.2 g/L); the total amount of HM proteins
149 was not affected significantly by any processing method.
150 Lactoferrin content of unpasteurized HM varied between 0.54 and 1.59 g/L (median: 0.82 g/L) and
151 was affected negatively by all the three pasteurization methods. A high reduction in HM lactoferrin
152 content was observed following both thermal pasteurization methods, with HoP having the greatest
153 effect (-87.5% after HoP and -83.5% after HTST). The difference between the two thermal methods
154 was not significant. On the contrary, HPP was found to better preserve HM lactoferrin content (-
155 24.7%). Secretory IgA content of unpasteurized HM varied between 2.1 and 3 g/L (median: 2.3
156 g/L) and was negatively affected by all pasteurization methods, but to a different extent. In
157 particular, only HPP led to a significant reduction (-35.8%) of sIgA content, with an almost double
158 reduction if compared to HTST (-23.3%).
159 In Figure 1, the protein profile of the five pooled HM sub-samples in non-reducing conditions is
160 reported; for one sample, the reducing protein profile is also displayed. For all the HM samples, the
161 reducing conditions were not suitable to visualize any difference in protein band abundance. When
162 non-reducing conditions were used, variations on specific HM protein band abundance were seen.
163 Moreover, these changes were common to all individuals, despite minor differences in the baseline
164 HM profile. Variations seemed more relevant for HoP, followed by HTST and, finally, by HPP.
165 In Figure 2, one individual protein profile was magnified to highlight the bands accounting for
166 visible changes. The identity of the proteins contained in these bands was assessed by comparison
167 with previous publications by our group (19–21): protein aggregates (mainly lactoferrin, band a)
168 seemed to be increased by all pasteurization methods, although to a different extent. On the
169 contrary, components of the immune system (bands b and c, containing heavy constant chain
170 subunits and polymeric receptor of immunoglobulin) and lactoferrin (band d) were decreased in all
171 pasteurized HM samples compared to raw HM, in variable amounts according to the processing
172 method used. For thermal methods only (HoP and HTST), the protein profile showed an increase of
173 two low molecular mass bands (band e and f), corresponding to beta casein fragments. These

174 shorter beta casein fragments have been always found following thermal pasteurization (HoP and
175 HTST) in our previous (19–21) and ongoing experiments, although we cannot discriminate if they
176 are native beta casein variants or a degradation product of the main beta casein form.
177
178 • *Microbiological analyses*
179 Each pasteurization method proved to comply with safety standards required by HMB guidelines.
180 Specifically, total bacterial count in all the raw HM samples was lower than 10^5 CFU/mL and
181 Enterobacteriaceae and *S. aureus* count were lower than 10^4 CFU/mL. Bacterial count was
182 undetectable in all the pasteurized HM samples, treated by HoP, HTST, or HPP.
183
184 *Discussion*
185 The availability of DHM provided by HMBs has been recently included among the milestones for
186 enteral nutrition of preterm infants developed in the last century (22). However, despite a huge
187 number of research efforts, current treatment of DHM is still unsatisfactory in terms of retention of
188 those bioactive components which are believed to mediate the beneficial clinical effects of HM in
189 preterm infants, such as the reduction of necrotizing enterocolitis and the improvement of long-term
190 neurocognitive outcome (23,24). For this reason, current research is directed towards the
191 identification of novel pasteurization methods which would be capable to preserve HM bioactive
192 components without affecting microbiological safety. To date, a number of papers on the effects of
193 innovative processing techniques for DHM pasteurization have been published and the results of
194 these papers have been summarized in recent reviews (13–16). Evidences on the impact of
195 innovative technologies are currently being evaluated by several research groups (18,19,25,26). The
196 assessment of new pasteurization technologies is hampered by the increase in parameters to be
197 monitored, others than time and temperature, especially for HPP, which is being tested in a high
198 variety of pressure settings (26). In an effort to elucidate the relative advantages of the most
199 promising innovative technologies over the traditional HoP method, we designed a study to

compare the effects of HoP, as performed in HMBs, vs. HTST, performed with a patented proprietary device validated for treating HM, and continuous HPP on individual HM samples. All previous reports on the issue (26–28) were conducted by simulating the processing treatments on small HM volumes or even on single protein fractions, but no report to date used real HMB conditions for processing whole individual HM samples to compare the three methods. In the tested conditions, none of the processing methods affected the total amount of proteins, thus confirming previous observations (13–15).

On the other hand, some differences were found in the quantity of specific proteins, as assessed by ELISA and by protein electrophoresis: HTST was found to better preserve the original content of sIgAs, in comparison to HPP, to a higher extent in comparison to the standard processing method. These results are in contrast with previous reports (28), which found a double rate of degradation of IgAs after HoP and HTST, as simulated on very low amounts (40 µl) of skimmed HM. It is to mention that one of the reasons for this discrepancy is that, in our study, HTST is performed by a prototyped continuous flow device, rather than in batch. Through this technique, the damage caused by heat is minimized by forming a thin layer of milk flowing in a continuous tube system, requiring very short time to reach the operating temperature, and, equally relevant, minimizing times for cooling down the milk, which is not the case in batch processes.

The rate of retention of sIgAs in our study for HoP and HPP was similar to that reported by Permanyer and colleagues (29). An opposite trend was found, in the tested conditions, for another important antibacterial factor, lactoferrin. Lactoferrin content was strongly reduced by both thermal pasteurization techniques, as measured by ELISA tests, while it was better preserved by HPP. Recently, Wesolowska and colleagues (26) found that HP pasteurization at 600 MPa for 10 min allowed retaining 55% of the original lactoferrin content. In the current study, HPP at same pressure for 3 min was enough to bring microbial growth to undetectable level, thus retaining almost 65% of the original lactoferrin content in raw HM. The tendency of native HM lactoferrin to be denaturated following pasteurization was investigated (27), and the kinetics of HM lactoferrin denaturation as a

consequence of HPP reported in that study are in accordance to our present data. Nevertheless, efficacy of HPP for 3 min at 600 MPa in guaranteeing microbial safety should be better confirmed in future by inoculation and validation studies, such as those performed for the HTST prototype used in the present study (19).

When evaluating protein band intensity by protein electrophoresis under reducing conditions, the lactoferrin band intensity was comparable among all the samples, including raw milk. When running the tests in non-reducing conditions, as in other reports (19,20,27), we observed that conformational changes of lactoferrin occurred, driven by the thermal treatments, resulting in an increase of high molecular weight aggregates, causing an apparent decrease of the original lactoferrin band. This phenomenon occurred mostly in HoP-treated milk, while it seemed to be less pronounced following HPP and HTST. This observation highlights the limits of techniques based on antibody recognition to detect aggregated complexes of lactoferrin, thus limiting the exact quantification of the absolute lactoferrin content in HM treated by thermal methods. The effect of conformational changes of lactoferrin on its bioavailability and bioactivity remains to be assessed.

In conclusion, the results of the present study show that HTST and HPP affect differently some of the bioactive HM components involved in immune response and antibacterial activity. In addition, both methods demonstrate to better preserve the original protein profile of raw milk, compared to standard HoP. Further studies should be aimed at characterizing residual protein bioactivity in HM treated with the two different pasteurization methods.

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References

1. American Academy of Pediatrics - Section on Breastfeeding. Breastfeeding and the use of human milk. Pediatrics. 2012;129:e827-41.
2. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013;60:49–74.
3. Committee on Nutrition - Section on Breastfeeding - Committee on Fetus and Newborn. Donor Human Milk for the High-Risk Infant: Preparation, Safety, and Usage Options in the United States. Pediatrics. 2017;139:e20163440.
4. Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, Lee HC. Impact of Donor Milk Availability on Breast Milk Use and Necrotizing Enterocolitis Rates. Pediatrics. 2016;137:e20153123–e20153123.
5. Sullivan S, Schanler RJ, Kim JH, et al. An exclusively human milk-based diet is associated with a lower rate of necrotizing enterocolitis than a diet of human milk and bovine milk-based products. J Pediatr. 2010;156:562-7.e1.
6. Arslanoglu S, Moro GE, Bellù R, et al. Presence of human milk bank is associated with elevated rate of exclusive breastfeeding in VLBW infants. J Perinat Med. 2013;41:129–31.
7. Agência Nacional de Vigilância Sanitária - Brasil. Banco de Leite Humano: Funcionamento, Prevenção e Controle de Riscos [Internet]. 2008 [cited 2019 Feb 8]. Available from: http://www.redeblh.fiocruz.br/media/blhanv2008.pdf
8. Arslanoglu S, Corpeleijn W, Moro G, et al. Donor human milk for preterm infants: current evidence and research directions. J Pediatr Gastroenterol Nutr. 2013;57:535–42.
9. Hartmann BT, Pang WW, Keil AD, Hartmann PE, Simmer K. Best practice guidelines for the operation of a donor human milk bank in an Australian NICU. Early Hum Dev. 2007;83:667–73.

10. Human Milk Banking Association of North America (HMBANA). Guidelines for the establishment and operation of a donor human milk. HMBANA. 4th Edition 2019. https://www.hmbana.org/our-work/publications.html. Accessed July 29, 2019.
11. Italian Association of Human Milk Banks, Arslanoglu S, Bertino E, et al. Guidelines for the establishment and operation of a donor human milk bank. J Matern Fetal Neonatal Med. 2010;23:1–20.
12. National Institute for Health and Care Excellence. Donor milk banks: service operation. NICE Guideline 2010. Last update July 2018. https://www.nice.org.uk/guidance/cg93. Accessed July 29, 2019
13. Moro GE, Arslanoglu S. Heat treatment of human milk. J Pediatr Gastroenterol Nutr. 2012;54:165–6.
14. Peila C, Moro GE, Bertino E, et al. The effect of holder pasteurization on nutrients and biologically-active components in donor human milk: A review. Nutrients. 2016;8:477.
15. Picaud J-C, Buffin R. Human Milk—Treatment and Quality of Banked Human Milk. Clin Perinatol. 2017;44:95–119.
16. Peila C, Emmerik NE, Giribaldi M, et al. Human Milk Processing: A Systematic Review of Innovative Techniques to Ensure the Safety and Quality of Donor Milk. J Pediatr Gastroenterol Nutr. 2017;64:353–61.
17. Sousa SG, Delgadillo I, Saraiva JA. Human Milk Composition and Preservation: Evaluation of High-pressure Processing as a Nonthermal Pasteurization Technology. Crit Rev Food Sci Nutr. 2016;56:1043–60.
18. Escuder-Vieco D, Espinosa-Martos I, Rodríguez JM, et al. High-temperature short-time pasteurization system for donor milk in a human milk bank setting. Front Microbiol. 2018;9:926.
19. Giribaldi M, Coscia A, Peila C, et al. Pasteurization of human milk by a benchtop High-Temperature Short-Time device. Innov Food Sci Emerg Technol. 2016;36:228–33.

20. Baro C, Giribaldi M, Arslanoglu S, et al. Effect of two pasteurization methods on the protein content of human milk. Front Biosci. 2011;3:818–29.
21. Giribaldi M, Ortoffi M., Giuffrida MG, et al. Effect of prolonged refrigeration on the protein and microbial profile of human milk. Int Dairy J. 2013;31:121–6.
22. Van Goudoever JB. Nutrition for Preterm Infants: 75 Years of History. Ann Nutr Metab. 2018;72:25–31.
23. Patel AL, Kim JH. Human milk and necrotizing enterocolitis. Semin Pediatr Surg. 2018;27:34–8.
24. Schneider N, Garcia-Rodenas CL. Early nutritional interventions for brain and cognitive development in preterm infants: A review of the literature. Nutrients. 2017;9:187-192.
25. Donalisio M, Rittà M, Francese R, et al. High Temperature—Short Time Pasteurization Has a Lower Impact on the Antiviral Properties of Human Milk Than Holder Pasteurization. Front Pediatr. 2018;6:1–7.
26. Wesolowska A, Sinkiewicz-Darol E, Barbarska O, et al. New Achievements in High-Pressure Processing to Preserve Human Milk Bioactivity. Front Pediatr. 2018;6:1–10.
27. Mayayo C, Montserrat M, Ramos SJ, et al. Kinetic parameters for high-pressure-induced denaturation of lactoferrin in human milk. Int Dairy J. 2014;34:246–52.
28. Mayayo C, Montserrat M, Ramos S, et al. Effect of high pressure and heat treatments on IgA immunoreactivity and lysozyme activity in human milk. Eur Food Res Technol. 2016;242:891–8.
29. Permanyer M, Castellote C, Ramírez-Santana C, et al. Maintenance of breast milk Immunoglobulin A after high-pressure processing. J Dairy Sci. 2010;93:877–83.

Figure legends

Figure 1: NuPAGE total protein profile of human milk (HM) after different pasteurization processes. Colloidal Coomassie brilliant blue stained. Each image (sample) is representative of one pooled HM sub-sample. Std: mass markers Mark12 (Thermo Fisher Scientific). A: unpasteurized HM; B: holder pasteurized HM; C: high pressure processed HM; D: high-temperature short-time pasteurized HM. Sample 5 protein profile is represented in both absence (non-reducing) and presence (reducing) of Dithiotreitol.

Figure 2: NuPAGE total protein profile of human milk (HM) after different pasteurization methods. Colloidal Coomassie brilliant blue stained. Bands a-f were identified in previous studies. (19–21). Std: mass markers (kDa) Mark12 (Thermo Fisher Scientific). A: unpasteurized HM; B: holder pasteurized HM; C: high pressure processed HM; D: high-temperature short-time pasteurized HM.