Impact of HTST pasteurization of human milk on the kinetic of digestion of macronutrients after in vitro dynamic digestion

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Introduction

Donor human milk (HM) represents the best alternative when mother's own milk is not available, a common occurrence in Neonatal Intensive Care Units. Heat treatment of donor HM is mandatory for safety reasons: Holder pasteurization (HoP, 62.5°C-30') is recommended by all HM bank guidelines. A recent study [1] has demonstrated that HoP affects the digestion profile and behavior of several HM components. High Temperature-Short Time pasteurization (HTST, 71°C-15") is currently under evaluation as a promising alternative technology to limit the denaturation of some biological compounds of raw HM and, very recently [2], it was shown to better preserve the milk antiviral activity with respect to HoP

Objectives

The aim of the present work was to assess whether the different types of pasteurization (HoP, HTST) impact the digestive kinetics of human milk during in vitro dynamic digestion.

Methodology

The experimental design is summarized in **Fig. 1**. HoP was performed by using the Human Milk Bank equipment (Metalarredinox, Italy), and HTST by a patented proprietary small-scale device [3].
Raw, HoP and HTST human milk were subjected to gastrointestinal digestion using DIDGI® system [4]; samples were characterized for:

- Particle size distribution (PSD) on undigested and gastric samples (Mastersizer 2000 laser light scattering with two laser sources, Malvern Instruments);
- Confocal microscopy: The microstructure was observed using Nikon C1Si confocal laser scanning microscopy (CLSM) on inverted microscope TE2000-E (Nikon, Champigny-sur-Marne, France) as previously described by Bourlieu et al. (2015). FastGreen® and Lipidtox® (Thermo-Fisher Scientific) were used for staining simultaneously proteins (Blue) and apolar lipids (Green), respectively.
- Protein profile: (reducing and non reducing NuPAGE® 4-12%, Life Technologies) and MS identification of differential bands (Q Exactive™Mass Spectrometer, Thermo Scientific);
- Amino acid (AA) profile on undigested and intestinal samples (cation exchange chromatography, Biochrom 30 automatic AA analyzer, Biochrom);
- Triglyceride content (thin layer cromatography and densitometry analysis by Image Quant TL (GE Healthcare).

Statistical analyses were performed using the PAST3 software package.

Results

- Both pasteurization methods led to heat-induced protein aggregates (blue dots), both in the soluble phase and at the interface of the HM fat globule membrane. The protein-fat interaction phenomena, which could be seen also in raw human milk, seems to be more relevant following HoP treatment, with respect to HTST **(Fig. 2)**
- During gastric digestion, both pasteurization methods modified PSD, as compared to RHM **(Fig. 3)**;
- Caseins were rapidly hydrolyzed in the gastric phase. Lactoferrin was hydrolyzed faster in the pasteurized samples in comparison to Raw HM, in which lactoferrin was more resistant to gastro-intestinal digestion. In particular, the kinetics digestion of iGa was statistically similar between RHM and HTST milk **(Fig. 4)**;
- Heat-treatments, consequently, affected the intestinal release of AA, and a **significantly higher bioaccessibility** ($p < 0.05$) **of AA was found for HTST,** as compared to HoP **(Fig. 5)**;
- **No difference** were found about **Lipolisys** between HoP and HTST **(Fig. 6).**

Conclusion

This work provides the first important evidences on the **differential impact of HoP and HTST pasteurization techniques on bioaccessibility OF aa AND IgA of DHM** for preterm newborns.

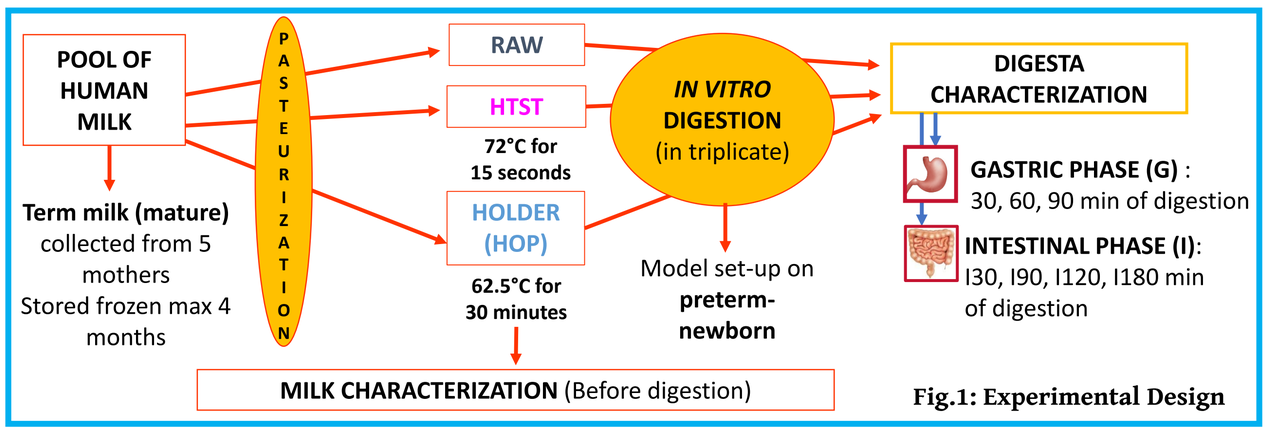

Fig.1: Experimental Design

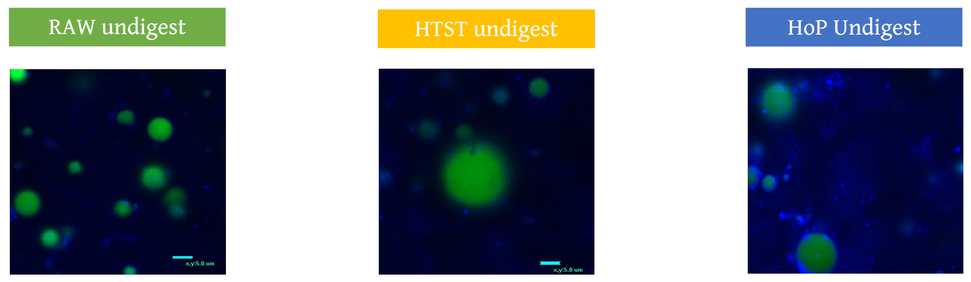

Fig. 2: Confocal microscopy, protein aggregation in blue

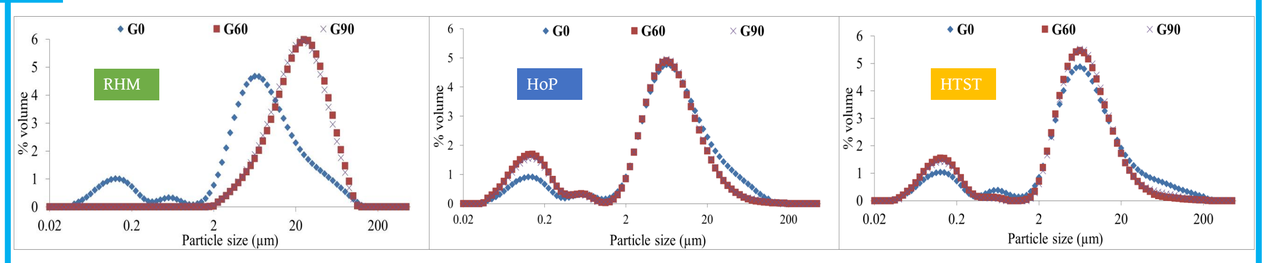

Fig. 3: Size distributions of Gastric Digests particles of Raw and Pasteurized HM

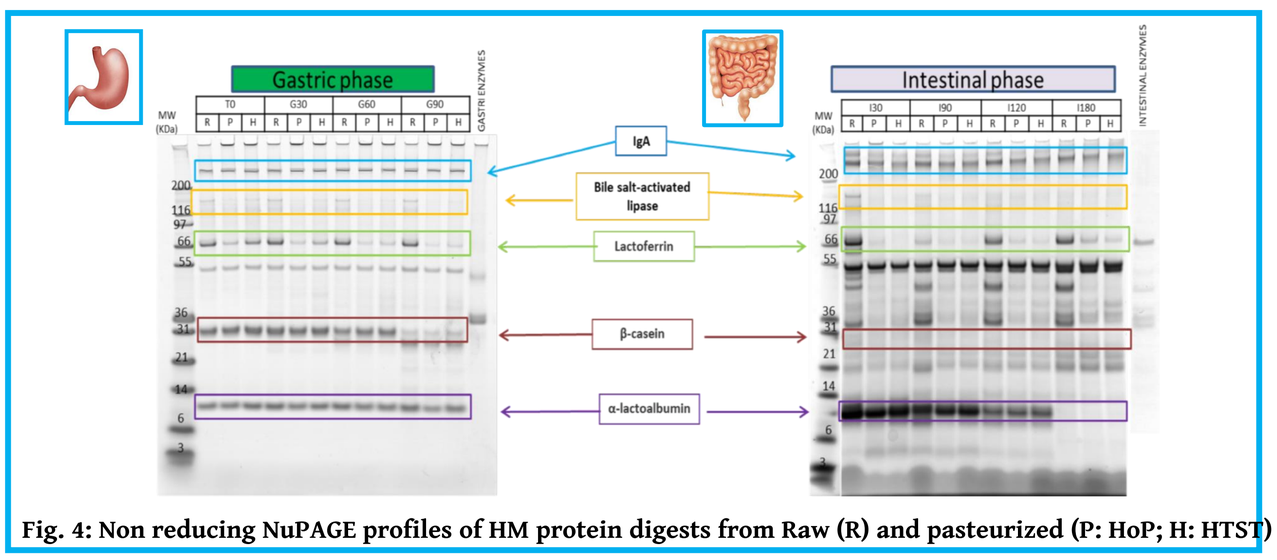

Fig. 4: Non reducing NuPAGE profiles of HM protein digests from Raw (R) and pasteurized (P: HoP; H: HTST)

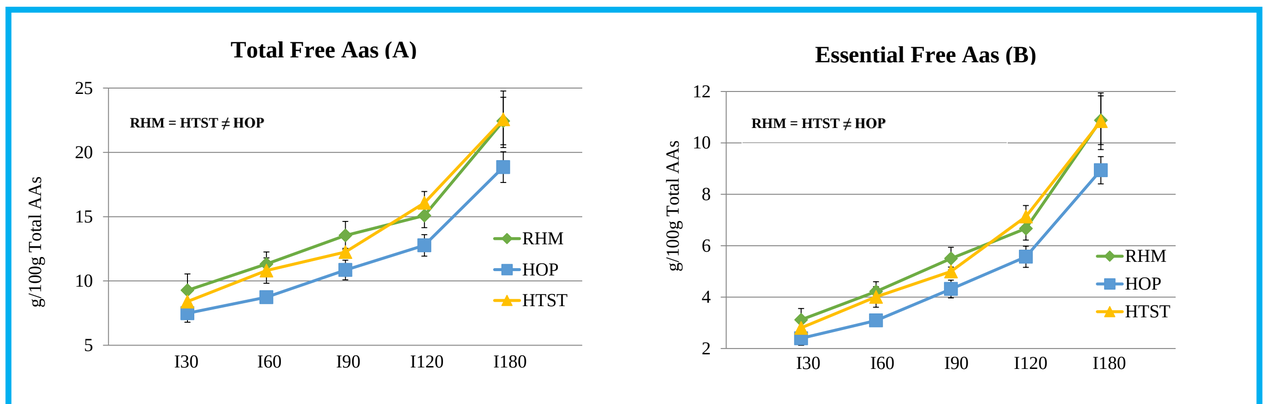

Fig. 5: % Free AA over Total sum (A) and Essential AAs (B).

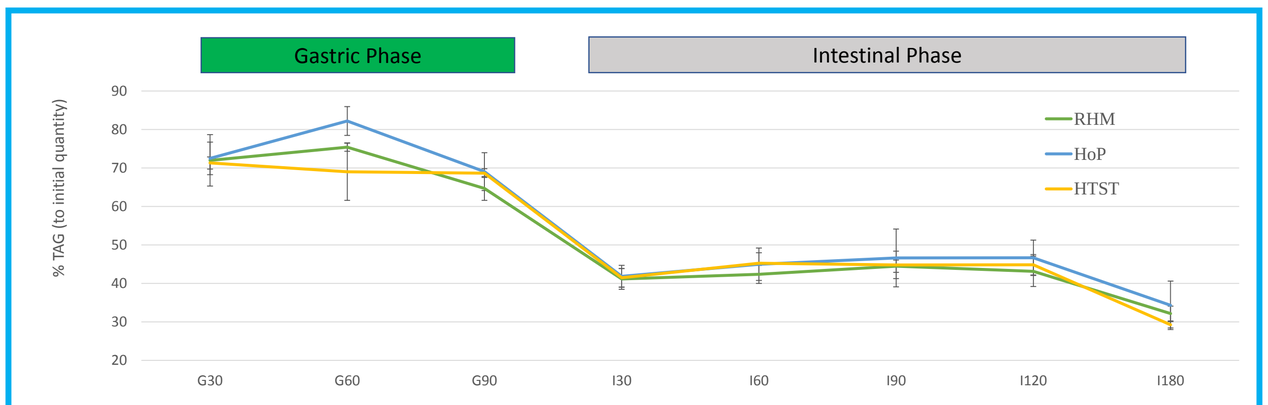

Fig. 6: % Free TAG over initial quantity during Gastric and Intestinal phases.

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