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Pasteurization of human milk by a benchtop High-Temperature Short-Time device

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ABSTRACT

A new small-scale continuous-flow High-Temperature Short-Time (HTST) pasteurizer has been designed for treating human milk. The efficacy of the new HTST device was assessed on inoculated *Listeria monocytogenes*, *Staphylococcus aureus* and *Chronobacter sakazakii*, as well as on raw human milk bacteria. The milk biochemical quality after HTST pasteurization was assessed in comparison to a standard Holder pasteurization, by determining the secretory IgAs (sIgAs) content, the protein profile, lysozyme and the Bile Salt Stimulated Lipase (BSSL) activities. No pathogen or bacterial growth was detected after HTST pasteurization with the new instrument. Changes in the protein profile were observed in the milk pasteurized according to both processes. The sIgAs content and BSSL activity were significantly higher in the milk pasteurized with the new device than in the same milk treated by the standard Holder pasteurization. In conclusion, the new HTST apparatus: (i) can effectively pasteurize human milk with a better retention of sIgAs content and BSSL activity; (ii) comply to human milk banking safety requirements.

Industrial relevance: Currently, 210 active human milk banks are located in Europe (and 17 more are planned). The majority of the European banks still use Holder-based pasteurizers, which, despite efficacy in ensuring microbiological safety, are known to reduce/disrupt important nutritional and non-nutritional biological factors. Although already widely established in food industry, the advantages of HTST technology were tested only at small laboratory scale for human milk. The device tested in the present research was specifically designed to provide human milk banks with the technology they need to ensure a safe and lower-impact pasteurization process, that is suitable for processing different volumes of donations. The device can pasteurize up to 10 L of milk per hour, with a minimum volume of 100 mL. The system is designed to be cleaned-in-place (CIP) after each pasteurization run and sanitized immediately prior to the next use, being thus more suitable for treating pools of milk from different donors than milk from single donations. Italian and EU patents have been filed for the device, within a partnership between public research institutions, stakeholders (Italian association of donor milk banks), and a private company in the sector of dairy processing equipment. The device has achieved a Technology Readiness Level (TRL) 6 (Prototype demonstration in a relevant environment). The cost of the new device will be comparable to that of a typical human milk Holder pasteurizer.

1. Introduction

Thermal treatments are commonly applied for food processing, because of their ability to kill pathogens and inactivate potentially detrimental enzymes, such as lipases and proteases. The Holder pasteurization (HoP) method (62.5 °C for 30 min) is currently the recommended pasteurization method for human milk banks (HMBs), as it ensures that the human milk (HM) is microbiologically safe (Arslanoglu et al., 2010). However, heat processing, particularly under severe conditions, may give rise to chemical and physical changes that can impair the

Abbreviations: BSSL, Bile Salt Stimulated Lipase; CFU, Colon Forming Units; HTST, High-Temperature Short-Time; HoP, Holder pasteurization; HM, Human milk; HMB, Human milk bank; IHM, Inoculated human milk; OHM, Original human milk; PHM, Pasteurized human milk; RHM, Raw human milk; sIgAs, Secretory IgAs; SHM, Sterile human milk; TVC, Total Viable bacteria Count.

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organoleptic properties and reduce the content or bioavailability of some nutrients (Arslanoglu et al., 2013; Tully, Jones, & Tully, 2001). HM has an elevated biological value, thanks to the casein-to-whey ratio, high essential amino acid concentration and immunological components, such as immunoglobulins, lysozyme, and lactoferrin, which convey relevant antimicrobial properties (Andreas, Kampmann, & Mehring Le-Doare, 2015; Giribaldi et al., 2012). Thermal treatments may also cause the unfolding of milk fat globule membrane proteins and whey proteins, whose end products are typically associated with off-flavours (Contador, Delgado, García-Parra, Garrido, & Ramírez, 2015). The loss of some biologically active components, including immunological components, due to HoP, is a main limit to the spread of donor human milk (Moro & Arslanoglu, 2012; Tully et al., 2001). The maximization of the biological and nutritional quality of donor HM is considered a scientific and social priority: the ESPGHAN Committee on Nutrition has pointed out that "future research should focus on the improvement of milk processing in human milk banks, particularly of heat treatment" (Arslanoglu et al., 2013).

In a previous study by our group (Baro et al., 2011), the HTST method (72 °C for 15 s) showed to better preserve, in comparison to HoP, the milk protein profile and some of the key active components of HM, with potential consequences on the availability of important nutritive compounds, such as fatty acids and available lysine.

In previous studies concerning the application of HTST to HM, other non-commercial devices were used, usually at a laboratory scale. Some authors used laboratory equipment, mainly consisting of stainless steel tubing systems submerged in thermostated water baths, through which HM was pumped (Dhar, Fichtali, Skura, Nakai, & Davidson, 1996; Goldsmith, Eitenmiller, Toledo, & Barnhart, 1983; Terpstra et al., 2007); others injected milk through a sterile water stream in a plate-type industrial heat exchanger (Goldblum et al., 1984); some research studies were conducted by directly heating and rotating small aliquots of milk, to simulate the typical thin-layering of dairy industry HTST devices (Goelz et al., 2009; Hamprecht et al., 2004). Several studies involved simply heating small aliquots (from 40 μL to 4 mL) of HM in a bulk process (Mayayo et al., 2014; Mayayo et al., 2016; Silvestre et al., 2006, 2008). Moreover, variable heating times (5–15 s) and temperatures (71–75 °C) were adopted. All the reported processes, with the exception of that of Goldblum et al. (1984), are substantially different from industrial HTST processes. However, the introduction of HTST into the HMB routine has not been possible to date, due to the lack of specific low-volume designed instrumentation. In most of studies concerning the HTST processing of HM, quantitative comparisons, with respect to standard pasteurization, have been described, but HoP was often simulated on small aliquots, rather than being performed according to real HMB-implemented protocols, thus representing a possible bias for the generalization of the comparison with novel technologies (Peila et al., 2015).

When projecting, creating, testing and patenting a new type of low-volume HTST pasteurizer (Cavallarin et al., 2015), it was considered that: i) it was intended for HMBs, and thus consistency with guideline requirements was mandatory; ii) the dairy industry HTST standards (72 °C holding temperature for 15 s holding time) had to be fulfilled; iii) temperature control had to be ensured by means of adequate probes; iv) a comparison with the HoP process had to be made using the HMB implemented device.

The present research is aimed at reporting the efficacy of a new, low-volume, continuous flow commercial HTST pasteurizer on HM quality in terms of: (i) bacterial inactivation, and (ii) preservation of the main immunological and nutritional components.

2. Materials and methods

2.1. Preliminary tests

In order to verify the pasteurization process, a preliminary experiment was run. A bovine milk sample was pasteurized and the alkaline phosphatase and peroxidase activities were determined in the treated sample. Alkaline phosphatase is a heat sensitive enzyme in milk that is used as indicator of pasteurization. If milk is properly pasteurized, alkaline phosphatase is inactivated. Lactoperoxidase is one of the most heat-stable enzymes found in milk and it is preserved after a correct HTST pasteurization. The raw bovine milk was purchased from a local automatic distributor, collected in a sterile Pyrex bottle, delivered refrigerated to the lab and stored refrigerated until it was processed. The milk (1 L) was divided into two aliquots: one was kept refrigerated until it was analysed; the other was subjected to HTST pasteurization by means of the new instrument (described in Section 2.3), collected in a sterile Pyrex bottle and stored refrigerated until the analysis. Alkaline phosphatase activity was tested by means of the enzymatic hydrolysis of p-nitrophenol phosphate, which yields p-nitrophenol and inorganic phosphate (ISO/TS 6090, 2004). Peroxidase activity was determined by means of Storch's peroxidase test, which measures the oxygen transfer from hydrogen peroxide to other readily oxidisable substances (Council Directive 92/46/EEC).

2.2. Sample collection and pooling of specimens

The HM samples were obtained from the HMB of the Città della Scienza e della Salute in Torino, Italy, from eight healthy donor mothers. The donors cleaned their hands and breasts according to the HMB guidelines. The milk specimens were collected in sterile bisphenol-free polypropylene bottles (Flormed, Naples, Italy) using a breast pump and stored, by the HMB, at −20 °C until processed.

The HM samples were pooled separately for the two experiments, which were referred to as Experiment 1 and Experiment 2. Panel A and panel B in Fig. 1 show the experimental workflows for Experiment 1 and Experiment 2, respectively.

In Experiment 1, frozen samples from individual HM donors were thawed overnight in a refrigerator, and then in tap water, and were pooled to achieve a final volume of about 2 L in a sterile Pyrex bottle. They were then mixed carefully, divided into 100 mL aliquots and placed in sterile bisphenol-free polypropylene bottles. One aliquot of original HM (OHM) was used directly to determine the HM background microflora. The remaining aliquots were pasteurized intensively in a water bath for 1 h at 63 °C (prolonged Holder pasteurization) to kill all of the existing vegetative forms of microorganisms (SHM). One aliquot was kept for about 20 h in the refrigerator, and then analysed to verify the absence of microbial contaminants. The other aliquots were immediately frozen and used later for inoculation (IHM) (Section 2.4.3).

In Experiment 2, samples from individual HM donors were obtained frozen from the HMB, thawed overnight in a refrigerator, and then in tap water, and were pooled to achieve a final volume of about 400 mL in a sterile Pyrex bottle. They were mixed carefully and divided into three sterile bisphenol-free polypropylene bottles. Two aliquots were subjected to standard HoP, in two separate trays, in the HMB facilities. The two samples were processed in the same batch and differed only for their position in the pasteurizer. One aliquot was subjected to HTST pasteurization using the new instrument. The Raw (RHM), Holder (HoP-HM) and HTST (HTST-HM) pasteurized samples were kept frozen at −20 °C until analysis, with the exception of 10 mL per sample, which was kept refrigerated for about 20 h before being used for microbial screening.

2.3. Pasteurization equipment

HoP was performed in an HM pasteurizer (Metallarredinox, Zingonia-Verdellino (Bg), Italy) located in the HMB of the Città della Scienza e della Salute in Torino, Italy. A patent pending HTST-based proprietary system (Giada s.r.l., Villafranca Piemonte (To), Italy) (Cavallarin et al., 2015), specifically created for use in HMBs, was used for HM pasteurization at 72 °C for 15 s. The new instrument is a bench-top device