Impact of Holder pasteurization and high-pressure processing on human milk components

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Background

Human milk provides thousands of distinct bioactive molecules that protect babies against infection and inflammation. Human milk also contributes to immune maturation, organ development, and a healthy microbial colonization.[1] However, breastfeeding of preterm babies is sometimes difficult or practically unfeasible. Therefore, human milk from milk banks represents a good alternative, given that this product contains all nutrients needed by preemies for their complete development.[2]

Given that the health of these babies is rather fragile, donor human milk must be pathogen-free, while keeping an optimal nutritional value. Holder pasteurization, consisting in heating milk to 62.5°C in a water bath, is the most common method for inactivating viruses and bacteria in milk.[3] However, pasteurization is inefficient at destructing bacterial spores and is detrimental to the bioactivity of human milk.[4]

Studies have demonstrated that pasteurization by high-pressure processing (HPP), a non-thermal procedure, allows the retention of milk nutritional and immunological properties while mild heating (30-37°C) of the product and repetition of pasteurization cycles destroys bacterial spores.[5]

[1]Ballard O *et al*. Pediatr Clin North Am, 2013; [2]Wight, NE *et al*. J Perinatol, 2001; [3]Updegrove K *et al.* Midwifery Womens Health, 2013; [4]Koenig A *et al*. J Hum Lact, 2005; [5]Demazeau G *et al.* WO2014064385A1.

Objectives: To determine optimal parameters for HPP treatment of human milk, and to compare the effects of Holder pasteurization and HPP on bacterial load and on nutritional and immunological component retention.

Methods

Optimization of HPP parameters-preliminary data

We have determined that the use of pressures ranging from 300 to 500 MPa for 6 to 15 minutes allows efficient destruction of bacteria while preserving human milk IgA.

HPP assays

Lot #	Pressure (MPa)	Cycle time (min)	HPP cycles	Initial milk temperature (°C)
1-2	330	10	1 to 4	4°C or 37°C
3-8	425	6	4	4°C or 37°C

Analysis

Bacterial counts and nutritional values were analysed. Levels of IgA, IgG, IgM, lysozyme, lactoferrin, and lipase were determined. Untreated fresh milk samples and pasteurized milk samples were analysed concomitantly.

Results

TABLE 1 : Bacterial load (CFU/mL) in HPP-treated and pasteurized breast milk samples

Lots #	Fresh milk samples	HPP Parameters								Pasteurized samples
		330 MPa / 10 minutes / unheated				330 MPa / 10 minutes / heated to 37°C				
		1 cycle	2 cycles	3 cycles	4 cycles	1 cycle	2 cycles	3 cycles	4 cycles	
1	N/A*	63.3	23.3	26.7	16.7	20.0	3.3	3.3	0	383.3
2	26 200	3.3	0	0	0	0	0	0	0	26.7
		425 MPa / 6 minutes / 4 cycles								
		Unheated				Heated to 37°C				
3	6 950	0				0				3
4	37 300	493				213				1448
5	5 816	0				0				3
6	3 683	3				0				0
7	573	3				0				37
8	9 025	10				3				163

*N/A: Not available; CFU = colony forming units.

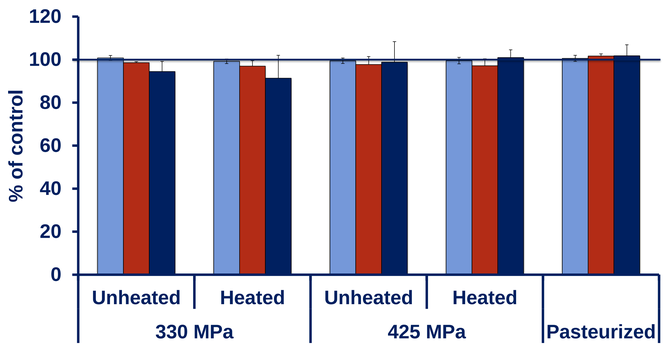

Figure 1: Nutritional values of HPP-treated and pasteurized breast milk samples. Results are presented as means ± SEM of percentage of control (set to 100%) Untreated milk samples contained: energy: 64.4 ± 4.2 Cal/100 mL; lipids: 3.2 ± 0.4 g/100 mL; proteins: 1.2 ± 0.5 g/100 mL; n = 2-8.

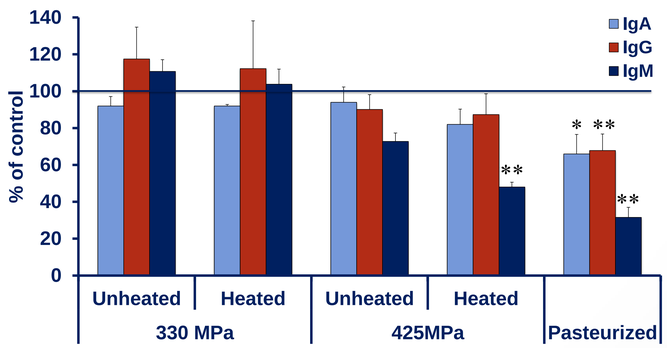

Figure 2: gA, IgG and IgM contents in HPP-treated and pasteurized breast milk samples. Results are presented as percentages of control (means ± SEM). Untreated milk samples contained: IgA: 342.1 ± 81.4 µg/mL; IgG: 20.5 ± 2.4 µg/mL; IgM: 7.1 ± 2.5 µg/mL. IgA, IgG, and IgM levels are significantly lower in pasteurized breast milk samples than in fresh milk samples (*p<0,05; **p<0,001). IgM content is also lower in heated and HPP-treated samples (425 MPa) than in fresh milk samples (**p<0,001); n= 2-8.

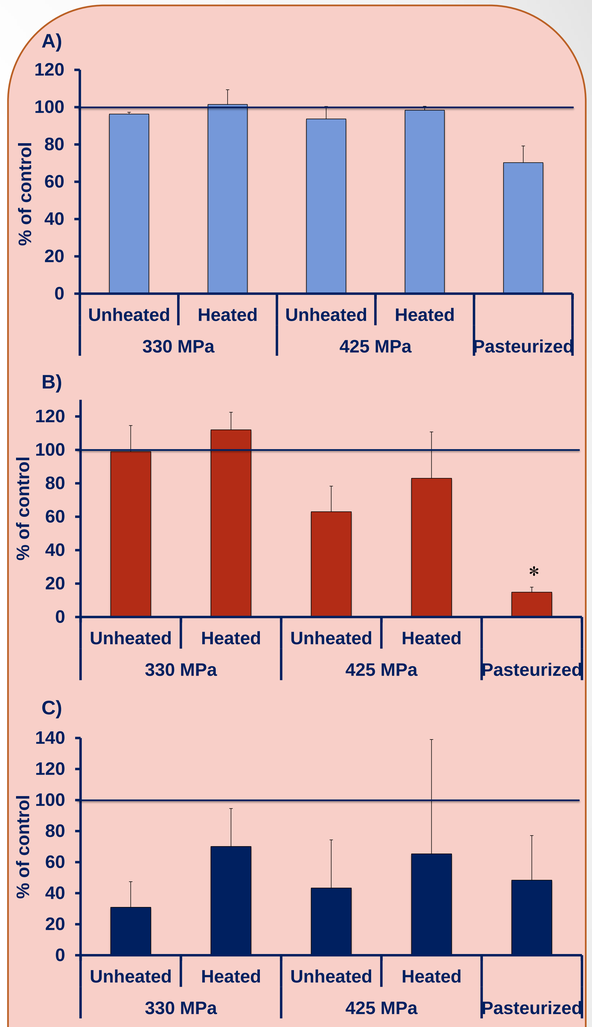

Figure 3: Content in lysozyme (A), lactoferrin (B), and lipase (C) in HPP-treated and pasteurized breast milk samples. Results are presented as percentages of controls (means ± SEM). Untreated milk samples contained: lysozyme: 47 495.7 ± 16 237.1 U/mL, lactoferrin: 2.4 ± 0.4 mg/mL, lipase: 28.9 ± 19.1 pg/mL. Levels of lactoferrin are significantly lower in pasteurized breast milk samples than in fresh milk samples (p<0,05); n = 2-8.

Conclusions and Perspectives

HPP treatment of breast milk appears to be a good alternative to pasteurization. This cold pasteurization method allows a more efficient destruction of sporulating bacteria such as *Bacillus cereus*, thereby enhancing the quality of breast milk lots available to hospitals. Moreover, a higher retention of nutritional factors, immunoglobulins and lactoferrin is observed in HPP-treated breastmilk samples compared to pasteurized breast milk samples. No significant differences were observed between HPP-treated milk samples and fresh milk samples regarding lysozyme and lipase, suggesting that HPP treatment does not adversely affect these factors. Analysis of residual cytomegalovirus infectivity and levels of some cytokines present in breast milk could provide further information on the comparative analysis of the two human milk pasteurization methods.