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Effect of thermal pasteurisation and high-pressure processing on immunoglobulin content and lysozyme and lactoperoxidase activity in human colostrum

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ABSTRACT

Human milk, and particularly human colostrum, is the gold standard for newborn nourishment. Colostrum contains the highest concentration of immune factors, being the most potent immune booster known to science. In this work, we investigated Holder pasteurisation and high-pressure processing (HPP) effects on colostral IgA, IgM, IgG, lysozyme and lactoperoxidase. The amount of Igs was significantly decreased after Holder pasteurisation (20%, 51% and 23% for IgA, IgM and IgG, respectively), but fully preserved after HPP at 200 and 400 MPa. HPP at 600 MPa for 2.5 min resulted in the maintenance of IgA and losses of IgM and IgG (21% for both). The pressure treatments at 600 MPa for 15 and 30 min led to similar or higher losses than pasteurisation. *D*-values (min) for Igs ranged from 4941 to 452 at 400 MPa and from 235 to 40 at 600 MPa. Lysozyme activity was lost after pasteurisation (decreased 44%) and maintained after HPP. Lactoperoxidase activity was not detected. As far as the authors are aware, this is the first study evaluating HPP effects on human colostrum.

1. Introduction

Breastfeeding is the recommended nutrition for newborns and infants, since human milk provides nutrients, bioactive components and immune factors. The unique composition of this complex fluid promotes adequate growth and benefits the infants in several ways; e.g. enhancement of host defences and prevention of many infectious diseases. Human milk composition varies over the course of lactation, so that the lactation period is divided into three different stages: colostrum (1–5 days postpartum), transitional milk (6–15 days after birth) and mature milk (after 15 days) (Sousa, Delgadillo, & Saraiva, 2013). Colostrum is a richer source of nutrients and immune factors compared to mature milk, being the most potent natural immune booster known to science (Uruakpa, Ismond, & Akobundu, 2002). It contains a high concentration of proteins, mainly immunoglobulins (Igs) and lactoferrin, but also lysozyme and lactoperoxidase (LPO), and all possess antimicrobial activity. These "bioactive proteins" are relatively resistant against proteolysis in the gastrointestinal tract and, thus, contribute to the defence of breastfed infants against pathogenic bacteria and viruses.

There are several classes of immunoglobulins (IgA, IgM, IgG, IgE and IgD), and IgA, IgM and IgG are the major ones present in human milk. These specific Igs function by directly binding to specific microbial antigens, blocking binding and adhesion of pathogens, enhancing phagocytosis, modulating local immune function, and contributing to the infant's immune system development. IgA is present in human milk in its secretory form (sIgA) – a dimer of IgA linked together with a secretory component and a joining chain – and represents the predominant immunoglobulin fraction (>90%).

Lysozyme (EC 3.2.1.17), on the other hand, acts by degrading the outer cell wall of Gram-positive bacteria by hydrolysing β-1,4 linkages between N-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose residues. Besides causing bacterial cell wall lysis, lysozyme binds endotoxins (limiting its effect), increases IgA production, and contributes to macrophage activation (immunomodulatory effects). Lactoperoxidase (EC 1.11.1.7; LPO), in the presence of hydrogen peroxide (produced in small quantities by cells), catalyses the oxidation of thiocyanate (part of saliva) to antimicrobial compounds, such as hypothiocynate or higher oxyacids, which are able to kill both Gram-positive and Gram-negative bacteria.

Even though the mother's own milk is the gold standard for infant nutrition, sometimes the mother cannot adequately supply her child. In this case, donor human milk is a better alternative

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compared to bovine infant formulas, since donor milk has this great array of "bioactive factors" that cannot be mimicked by infant formula, making human breast milk so unique. However, because of the manner in which donor milk is collected, processed and stored in human milk banks (HMB) these prized properties, ascribed to the mother's own milk, are substantially diminished. The major process responsible for these losses is the low-temperature long-time (LTLT) pasteurisation, also known as Holder pasteurisation (62.5 °C for 30 min). This decreases IgA (up to 50%), IgG (34%) and lysozyme (up to 65%) contents, among others (Evans, Ryley, Neale, Dodge, & Lewarne, 1978; Heiman & Schanler, 2007), and completely destroys IgM in human milk (Ford, Law, Marshall, & Reiter, 1977). Nevertheless, pasteurisation is a mandatory step to assure microbial safety of human milk. Hence, alternative pasteurisation technologies able to render a better retention of human milk properties would be of great value.

High-pressure processing (HPP) is a novel non-thermal or cold (under refrigeration temperature) pasteurisation technology that is being increasingly applied in food industries worldwide, primarily as an alternative to thermal treatment. This is due to HPP capacity of providing safe foods with similar characteristics to the raw unprocessed foods (fresher-tasting and with better nutritive and functional properties) (Ramirez, Saraiva, Pérez Lamela, & Torres, 2009). A prime example of HPP potential is the pressurised antibody-rich bovine colostrum beverage currently commercialised, that could not be accomplished by thermal pasteurisation (Sousa et al., 2013). There are only few studies concerning the effect of HPP on mature human milk, which report 100% IgA retention after HPP, at 400 MPa for 5 min, at 12 °C (Permanyer et al., 2010) and IgA and lysozyme activities of 85.6% and 106.9%, respectively, after a 400 MPa pressure treatment for 30 min, at 21 °C (Viazis, Farkas, & Allen, 2007).

The aim of this study was to investigate and compare the effects of HPP (200, 400 and 600 MPa for 2.5, 15 and 30 min, at 8 °C) and of a Holder-like thermal pasteurisation on total IgA, IgM and IgG contents, and lysozyme and LPO activities in human colostrum. To our knowledge, this is the first work that evaluates HPP effects on human colostrum.

2. Materials and methods

2.1. Sample collection

Human colostrum samples, from 11 healthy mothers attending Infante D. Pedro Hospital (Aveiro, Portugal), were collected into sterile colostrum containers, by manual or mechanical expression of the breast, using an electric breast pump for the mechanical expression. All the material was provided by Medela, Portugal. The samples were collected 1–4 days after term delivery and always in the morning after the first feed of the day. Exclusion criteria for the donor mothers were those followed by the Human Milk Banking Association of North America (HMBANA), except for the one that excludes women which have been in the United Kingdom for more than 3 months or in Europe for more than 5 years, since 1980 (Sousa et al., 2013). Milk collection was previously approved by the Ethical Committee of Infante D. Pedro Hospital and an informed consent was obtained from all the donor mothers.

After collection, colostrum was transported to the laboratory in ice in less than 30 min, immediately frozen with liquid nitrogen and stored at −20 °C until thermal or pressure treatments were performed. On the day of the treatments, the samples were thawed, pooled to make a homogeneous batch and then divided into several aliquots for eleven different groups. These groups were the following: one group of raw untreated colostrum, one to be thermally pasteurised, and nine for the various HPP treatments (200, 400 and 600 MPa for 2.5, 15 and 30 min each). Along these treatments the samples were kept in ice slurry and at the end frozen in liquid nitrogen and stored at −20 °C for subsequent analysis. Samples were stored up to one week for lysozyme and LPO activity measurement and up to one month for immunoglobulin content analysis.

2.2. Thermal pasteurisation

The samples were thermally pasteurised by the Holder or LTLT pasteurisation method, according to the general HMBANA procedure (Sousa et al., 2013). Nine polypropylene tubes containing aliquots of 400 μl each were placed into a water bath at 62.5 °C, with one of them containing bovine milk and a thermocouple (instead of human colostrums) to estimate the time that colostrum would take to reach 62.5 °C. Once that temperature was reached, the samples were held in the water bath for 30 min. Following pasteurisation, the tubes were quickly cooled in an ice slurry. Human milk was used in this step, since the amount of colostrum collected was small, assuming a similar thermal conductivity for both. In addition, since the polypropylene tubes used were of a very small diameter (~0.5 cm) and the time of pasteurisation (30 min) was much higher than that need to reach 62.5 °C, this will have a negligible effect on the results obtained. Nevertheless, it was taken into account.

2.3. HPP treatments

The pressure treatments were carried out using a hydrostatic press from Unipress Equipment, Model U33 (Warsaw, Poland), with a pressure vessel of 100 ml (35 mm diameter and 100 mm height), surrounded by an external jacket, connected to a thermostatic bath to control the temperature. The unit has a maximum working pressure of 700 MPa and a working temperature between −20 °C and 100 °C. The pressure-transmitting fluid was a mixture of propylene glycol and water (60:40).

Nine distinct treatments were performed by combining three pressure levels with three treatment times: 200, 400 and 600 MPa for 2.5, 15 and 30 min each. The initial temperature of the pressure vessel was set to 8 °C. For each pressure treatment, colostrum was previously aliquoted to eight polypropylene tubes (400 μl each), which were then inserted into a small flexible plastic bag that was vacuum-sealed, placed inside another plastic bag and sealed again, with caution not to leave air inside the two bags.

2.4. Immunoglobulin content analysis

Immunoglobulins A, M and G were measured in human colostrum using specific human IgA, IgM and IgG ELISA kits (KOMA BIOTECH), respectively, according to the manufacturer's instructions. In order to describe the general procedures of the kits, the letter X will be used as a generic designation for any of the three Igs. The kits contained all of the required reagents and material for IgX quantitation: a pre-coated 96 well ELISA microplate (with antigen-affinity purified goat anti-human IgX), plate sealers, detection antibody (horseradish peroxidase conjugated antigen-affinity purified goat anti-human IgX), standard protein (human reference serum), assay diluent (1% bovine serum albumin), colour development reagents (tetramethylbenzidine and H_2O_2 solutions), stop solution (2 M H_2SO_4), and washing solution (phosphate buffered saline powder with 0.05% Tween-20, pH 7.4).

In each assay, 100 μl of blank, standard or colostrum samples were added to each well of the pre-coated microplate in duplicate and incubated at room temperature for 1 h. The standards were diluted following the manufacturer's recommended dilutions and