



# The effect of microwave-assisted heating on bioactive and immunological compounds in donor human milk

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## ABSTRACT

Low-Temperature Long-Time pasteurization (LTLT) is normally applied in donor human milk from Human Milk Banks (HMBs) to guarantee microbiological safety; however, this treatment can modify the protein structures, decreasing their beneficial effects. Thus, this study aimed to determine the impact of microwave-assisted heating on the concentration of key biological compounds in donor human milk to verify whether a microwave heating technique can be used as an alternative to LTLT pasteurization in Human Milk Banks. The concentrations of oligosaccharides, immunoglobulins, lactoferrin and fatty acids were monitored in raw donor milk and after processing to assess the impact of the microwave and LTLT treatments. The concentration of oligosaccharides was determined by HPAEC-PAD, immunoglobulins and lactoferrin were quantified using ELISA kits and fatty acids were quantified by gas chromatography. Oligosaccharides and fatty acids were not significantly affected ( $p > 0.05$ ) by LTLT and microwave processes; however, immunoglobulins and lactoferrin concentrations were better preserved when microwave-assisted heating was applied. For this reason, microwave-assisted heating can be considered a promising alternative to LTLT pasteurization of donor human milk in Human Milk Banks.

## 1. Introduction

Human milk (HM) is considered the optimal source of nutrition for feeding newborns and infants because it provides all the nutritional requirements necessary to promote healthy growth and development (Gartner, 2005; Léké et al., 2019). The presence of many immune compounds including immunoglobulins and cytokines, and biologically active components, such as lysozyme, lactoferrin, oligosaccharides, long-chain fatty acids and hormones, contribute to the maturation of the infant immune system and act as a protective barrier against pathogens (Agostoni et al., 2009; Comstock & Donovan, 2017; Fernandez et al., 2013; Newburg, 2005).

Although HM from the mother is considered the best option for infants, when it is unavailable, donor human milk from Human Milk Banks (HMBs) is the next best alternative to nourish preterm infants (Picaud & Buffin, 2017). To guarantee microbiological safety and extend the shelf-life of donor human milk, the heat treatment known as

Low-Temperature Long-Time (LTLT) pasteurization (62.5 °C for 30 min), is normally applied in most HMBs worldwide (Arslanoglu et al., 2010). However, LTLT pasteurization may decrease the concentration of some vitamins (e.g., vitamin B<sub>1</sub> and C) (Lima et al., 2020; Moltó-Puigmartí et al., 2011) and contribute to the denaturation of some proteins, which may lead to structural modifications for important bioactive proteins such as lactoferrin, lysozyme and IgA and hence eliminate beneficial bioactivities (Bjorksten et al., 1980; Guerra et al., 2018; H. K.; Lima et al., 2017; Peila et al., 2017; Wills et al., 1982).

According to Oliveira et al. (2016) and van Lieshout et al. (2020), LTLT pasteurization of human milk may cause changes in the protein structures, altering the gastric emptying of proteins, which eventually result in different postprandial absorption of amino acids and changes on the microbiota, epithelial physiology, and immune response. Moreover, changes in the protein structures may affect the infant's growth, neurodevelopment, hormonal regulation and appetite (Gan et al., 2018). Considering that the infants do not have the digestive and immune

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system fully developed, the preservation of protein structure is especially relevant in early life for pasteurized DHM fed infants.

Microwave-assisted heating is one of the most promising techniques being studied by our research group and others (Leite et al., 2019; Martysiak-Zurowska et al., 2019) as an alternative to traditional LTLT pasteurization used in HMBs. This technique provides some advantages, such as volumetric heating, high heat transfer rate, lack of intermediate heating fluid, preservation of food quality and fast processing times (Ahmed & Ramaswamy, 2020; Chandrasekaran et al., 2013; Martysiak-Zurowska et al., 2019). However, the microwave equipment needs to be well-dimensioned to avoid the occurrence of hot and cold spots that can affect the sensory characteristics and the microbiological stability of the milk (Atuonwu & Tassou, 2018; Chandrasekaran et al., 2013; Leite et al., 2019).

Malinowska-Pańczyk et al. (2019) reported that the application of microwave heating at 62.5 °C for 3 or 5 min (2450 MHz and 50 mL samples) provided similar inactivation of some heat-sensitive bacteria in human milk than LTLT pasteurization, with the advantage of significantly shortening the time to achieve microbial reduction. In our previous work (Leite et al., 2019), microwave heating (60 °C for 30 s) indicates a promising alternative to pasteurize human milk with a reduced residual alkaline phosphatase activity, below the value recommended by healthcare organizations, and achievement of safe microbiological quality.

Although some studies were investigating the effect of microwave-assisted heating on a large number of microorganisms and some proteins in human milk (Clare et al., 2005; Leite et al., 2019; Malinowska-Pańczyk et al., 2019; Martysiak-Zurowska et al., 2019; Nemethy & Clore, 1990; Quan et al., 1992), to our knowledge, no studies have reported the effect of this treatment on certain bioactive carbohydrates, such as oligosaccharides, and lipids in donor human milk. Human milk oligosaccharides (HMOs) are known to play an important role in the development of infants due to their prebiotic functionality, working as a growth substrate for beneficial bacterial species, such as bifidobacteria (Kobata, 2017). For instance, Yu et al. (2012) investigated the use of some individual HMOs as a supplement for in vitro growth of some types of bifidobacteria and reported that 2'-FL (2'-Fucosyllactose) can stimulate the proliferation of bifidobacteria longum ssp., leading to a decrease of *Escherichia coli* and *Clostridium perfringens* in the lower gut.

The acidic conditions imparted by probiotic bacteria in the large intestine of infants reduce the growth of many pathogenic microorganisms, protecting them from infections, and facilitating the establishment of beneficial gut microbiota (Chaturvedi et al., 2001; Jantscher-Krenn et al., 2012; Kobata, 2017; Manthey et al., 2014). Furthermore, some milk-derived fatty acids, such as conjugated linoleic acid, have beneficial biological effects for infants, including anticarcinogenic, antiatherogenic, antidiabetic and immune-stimulating properties (Gnädig et al., 2003; Oliveira et al., 2012). Ip et al. (1991) reported that supplementing the rat diet with doses of 0.5, 1, or 1.5% of conjugated linoleic acid, from the post-weaning until puberty, was sufficient to inhibit the growth of the mammary tumor by 32, 56 and 60%, respectively.

Given the importance of these biological components to promote healthy growth and development of infants, especially for premature infants in the Neonatal Intensive Care Unit, this work aimed to apply microwave-assisted heating in donor human milk at different conditions (60, 65 and 70 °C for 30, 15 and 10 s, respectively) to compare its effect with that of the conventional LTLT pasteurization (62.5 °C for 30 min) normally used in HMBs with respect to the preservation of oligosaccharides, immunoglobulins, lactoferrin and fatty acid concentrations in processed donor human milk.

## 2. Material and methods

### 2.1. Donor human milk collection

Human milk samples from lactating mothers with more than 15 days of lactation were collected from 10 volunteers at the Human Milk Bank (HMB) of the University Hospital of the University of São Paulo. The collection of human milk samples was approved by the Research Ethics Committee of the University of São Paulo (Process nº 1461/15, approved in 05/15/15 and 10/21/2016 by CEP and in 12/04/2016 by CONEP). All samples were delivered frozen to the Food Engineering Laboratory of Escola Politécnica of the University of São Paulo and kept frozen at −30 °C in a plasma freezer (349 FV, FANEM, São Paulo, Brazil) until further thawing, pooling and processing. The handling steps followed the recommendation of the National Institute for Health and Care Excellence guideline (NICE, 2010). After processing, the donor human milk was kept frozen at −30 °C until future analyses. The determination of oligosaccharides, immunoglobulins and lactoferrin concentrations in donor human milk before and after the treatments was carried out at the University of California, Davis, and the fatty acids profile at the Department of Biochemical and Pharmaceutical Technology of the University of São Paulo.

### 2.2. Microwave-assisted heating

As described in our previous publication (Leite et al., 2019), aliquots of 100 mL of donor human milk were submitted to different processing conditions (60, 65 and 70 °C for 30, 15 and 10 s, respectively), using a batch microwave reactor (Discover Reflux, CEM, Charlotte, USA) at 2450 MHz with a maximum power of 300 W. To a more reliable temperature monitoring, a fiber optic sensor (Fluoroptic STF-1M, Lumma-Sense Technologies, Santa Clara, USA) was inserted at the center of the liquid, connected to a data acquisition system (Luxtron 812, Lumma-Sense Technologies, Santa Clara, USA) that allowed temperature registration every 0.5 s to obtain the time-temperature history of the sample. Magnetic stirring in the reactor helped to provide uniform heating. Once the desired time-temperature binomial was achieved, the glass tube containing the milk sample was rapidly removed from the reactor and immersed in an ice-water bath until the temperature reached approximately 10 °C. Raw and processed samples were aliquoted and frozen at −30 °C in a plasma freezer (349 FV, FANEM, São Paulo, Brazil) until further analyses. Each process condition was applied in triplicate.

### 2.3. LTLT pasteurization

Aliquots of 100 mL of human milk were placed in a glass bottle with a screw cap and submitted to LTLT pasteurization in a thermostatic water bath (MA184, Marconi, Piracicaba, Brazil) at 62.5 °C for 30 min. After that, the samples were cooled immediately in ice water until the temperature reached approximately 10 °C. Once removed from the ice-water bath, the samples were aliquoted and frozen at −30 °C in a plasma freezer (349 FV, FANEM, São Paulo, Brazil) until further analyses. The thermal treatment was applied in triplicate.

The LTLT pasteurization was included in this study as a control, to compare its efficiency concerning the preservation of oligosaccharides, immunoglobulins, lactoferrin, and fatty acids with microwave-assisted heating.

### 2.4. Oligosaccharide isolation and purification

Oligosaccharides were isolated from raw and pasteurized milk samples as described by Sundekilde et al. (2012) and purified according to the method published by Salcedo et al. (2016), using nonporous graphitized carbon solid-phase extraction (GCC-SPE, Alltech, USA).

Briefly, 0.5 mL of raw or treated donor human milk was diluted with 0.5 mL of Milli-Q (18.2 MΩ-cm) water and centrifuged at 4000 × g for 30

min at 4 °C. The top fat layer was removed, and four volumes of a chloroform/methanol solution (2:1 v/v) were added. Then, the samples were centrifuged at 4000×g for 30 min at 4 °C and the upper layer containing oligosaccharides was collected and mixed with two volumes of pure ethanol. After overnight precipitation at 4 °C, the samples were centrifuged at 4000×g for 30 min at 4 °C and the supernatant (oligosaccharide rich-fraction) was collected and dried using a vacuum centrifuge. The dried oligosaccharides were resuspended in 1 mL of Milli-Q water and purified using nonporous graphitized carbon solid-phase extraction (GCC-SPE, Alltech, USA). After loading the oligosaccharide rich-solution onto GCC-SPE cartridge, the oligosaccharides were eluted with a solution of 40% acetonitrile and 0.1% trifluoroacetic acid (vol/vol) in water, then dried by vacuum centrifuge at 35 °C overnight before chromatographic analysis.

## 2.5. Oligosaccharides quantification by HPAEC-PAD

The dried oligosaccharide-rich fractions were reconstituted in 100 µL of Milli-Q water. The quantification of seven oligosaccharides, lacto-*N*-tetraose (LNT), lacto-*N*-neotetraose (LNnT), lacto-*N*-hexaose (LNH), 3'-sialyllactose (3'-SL), 6'-sialyllactose (6'-SL), lacto-*N*-fucopentaose I (LNFPI), and 2'-fucosyllactose (2'-FL), was carried out using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex ICS-5000+, equipped with an electrochemical detector cell containing a disposable gold working electrode and a pH-Ag/AgCl reference electrode (ThermoFisher Scientific, Waltham, MA). Chromatographic separation was conducted on a CarboPac PA200 analytical column (3 × 250 mm, ThermoFisher Scientific) and a CarboPac PA200 guard column (3 × 50 mm, ThermoFisher Scientific). Eluents consisted of water (A), 200 mM NaOH (B) and 100 mM NaOAc in 100 mM NaOH (C). The chromatographic gradient was programmed as follows: 0–10 min, 50% A 0% C; 10–35 min, B decreased from 50% to 45% and C increased from 0% to 5%; 35–40 min, B held at 45% and C increased from 5% to 10%. Each chromatographic run was preceded by a 5-min column wash at 100% B, followed by a 10-min equilibration at 50% B.

## 2.6. Quantification of immunoglobulins and lactoferrin by ELISA

The immunoglobulin (IgA, IgG and IgM) and lactoferrin concentrations in donor human milk before and after both microwave-assisted heating and LTLT pasteurization were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits, in triplicate. The ELISA kits n° E88-102, E88-104, E88-100 and E88-14 were used to quantify the levels of IgA, IgG, IgM, and lactoferrin, respectively, according to the manufacturer protocol (Bethyl Laboratories, Montgomery, Texas, USA). The milk sample absorbance was recorded using a spectrophotometer (SpectraMax M5, Sunnyvale, California, USA) at 450 nm.

## 2.7. Preparation of fatty acid methyl esters and quantification by gas chromatography

Fatty acids in raw and pasteurized donor human milk were extracted and methylated using International Standard Methods as described in ISO 14156 (ISO, 2001) and ISO 15884 (ISO, 2002), respectively. Fatty acids concentration were determined as described by Santos et al. (2020), using a gas chromatograph (430 GC, Varian, Netherlands) equipped with an automatic injector and flame ionization detector and a silica capillary column (SP-2560, Supelco, USA), with dimensions: 100 m length x 0.25 mm internal diameter. The operation conditions were: 1.5 mL/min column flow with the helium carrier gas, 280 °C detector temperature, 250 °C injector temperature, and a 50:1 split injection ratio. The oven temperature increased linearly from 75 °C to 240 °C at a rate of 3 °C/min, then held constant at 240 °C for 50 min.

The saturated (C10 (capric), C12 (lauric), C14 (myristic), C16

(palmitic) and C18 (stearic)), monounsaturated (C16:1 (palmitoleic) and C18:1 (oleic)), and polyunsaturated (C18:2 (linolenic) and C18:3 (α-linolenic)) fatty acids were identified by comparing the retention times of the peaks with those corresponding to the fatty acid standards (Rodrigues et al., 2007). All samples were analyzed in triplicate.

## 2.8. Statistical analyses

To determine the effect of LTLT pasteurization and microwave-assisted heating on the oligosaccharides, immunoglobulins, lactoferrin and fatty acid concentrations, statistical analysis was performed using one-way analysis of variance (ANOVA) in Minitab®18 (Minitab, Inc., USA). Following ANOVA, where interaction had a *p*-value < 0.05, Tukey's tests at the 95% confidence level were carried out to make multiple pairwise comparisons and determine the differences among the treatments for each compound (oligosaccharides, immunoglobulins, lactoferrin and fatty acids).

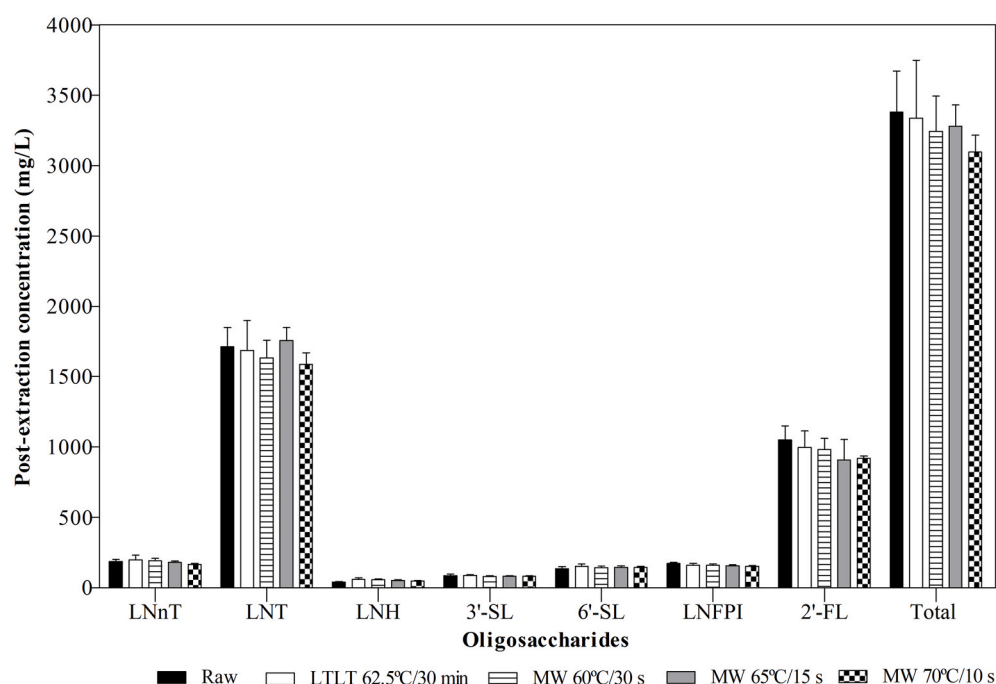
## 3. Results and discussion

As demonstrated in our previous publication (Leite et al., 2019), the use of batch microwave-assisted heating at a specific condition (60 °C for 30 s, 300 W, 100 mL samples) was sufficient to pasteurize donor human milk as evidenced by a 5-log reduction of *Salmonella* Typhimurium ATCC14028 and *Staphylococcus aureus* ATCC 25923 (Codex Alimentarius, 2004). In this study, the use of microwave-assisted heating at different conditions (60 °C for 30 s, 65 °C for 15 s and 70 °C for 10 s) was compared with conventional LTLT pasteurization (62.5 °C for 30 min) in terms of the preservation of oligosaccharides, immunoglobulins, lactoferrin and fatty acid concentrations in donor human milk. The results showed that microwave-assisted heating and LTLT pasteurization did not significantly affect the oligosaccharides and fatty acid concentrations in donor human milk; however, differences were observed in immunoglobulin and lactoferrin concentrations after the application of both LTLT and microwave techniques.

### 3.1. Post-extraction concentrations of oligosaccharides in raw and treated donor milk

The post-extraction concentration of human milk oligosaccharides (HMO) represents the amount measured after a thorough extraction, which included GCC-SPE. Ideally, SPE should be avoided before absolute milk oligosaccharide quantification because analyte recoveries are often significantly below 100% (Robinson et al., 2018; Xu et al., 2017). In this case, however, HMO analysis without SPE by HP AEC-PAD produced a large lactose peak that could not be resolved from those of some HMO structures (data not shown). Since the objective of this study could suitably be accomplished by comparing relative HMO abundance, as opposed to absolute concentration, we decided that performing GCC-SPE to minimize the lactose peak and resolve additional HMOs was worth the trade-off of reduced HMO recovery. Therefore, the HPAEC-PAD results presented in this study can be compared among processes to identify treatments that may degrade HMOs, but they should not be considered a reflection of the true HMO concentrations in the milk samples. Since the sample matrix was identical among the pasteurization and microwave-assisted heating, we expect very similar recoveries among all treatments, which allows the comparison of individual HMO abundance between treatment groups.

The post-extraction concentration of seven oligosaccharides (LNnT, LNT, LNH, 3'-SL, 6'-SL, LNFPI, and 2'-FL) in DHM (donor human milk) before and after both LTLT pasteurization and microwave-assisted heating are shown in Fig. 1. These seven HMOs were chosen as representative as they are some of the most concentrated in milk, and present all the key characteristics of the diversity of structures found in human milk (acidic, neutral, type 1, and type 2) (Bode, 2012; Huang et al., 2019; Ma et al., 2018). The total post-extraction oligosaccharide



**Fig. 1.** Post-extraction concentrations of oligosaccharides in donor human milk, both raw and processed by LTLT pasteurization (62.5 °C/30 min) and microwave-assisted heating (MWH) at different conditions (60 °C/30 s, 65 °C/15 s and 70 °C/10 s). The oligosaccharides were not significantly affected ( $p > 0.05$ ) by the processes (LTLT and microwave). LNnT = lacto-*N*-neo-tetraose, LNT = lacto-*N*-tetraose, LNH = lacto-*N*-hexaose, 3'-SL = 3'-sialyllactose, 6'-SL = 6'-sialyllactose, LNFPI = lacto-*N*-fucopentaose I and 2'-FL = 2'-fucosyllactose.

concentrations correspond to the sum of the seven oligosaccharides identified in DHM in each process (LTLT and microwave). The total and individual HMO concentrations were not significantly affected ( $p > 0.05$ ) by LTLT and microwave treatments, indicating the stability of the HMOs under the evaluated conditions.

Among the identified oligosaccharides, LNT and 2'-FL are those with the highest post-extraction concentration in raw and processed DHM, corresponding to approximately 51% and 31%, respectively, of the total HMO concentration in raw milk.

Hahn et al. (2019) also investigated the effect of LTLT pasteurization on HMO post-extraction concentration of human milk and, as observed in this study, reported that LTLT pasteurization did not affect their concentrations. There is no literature reporting the effect of microwave heating on DHM oligosaccharides. It is crucial to demonstrate that thermal processes do not affect the composition of oligosaccharides in donor human milk because HMOs have important prebiotic properties, contributing to the development of the infant's microbiota by selectively stimulating the growth of beneficial bacteria. Furthermore, oligosaccharides are recognized as receptors of pathogens, playing an important role in protecting vulnerable preterm infants from infection (Mudd et al., 2016).

### 3.2. Impact of LTLT pasteurization and microwave-assisted heating on the immunoglobulins and lactoferrin concentrations in donor human milk

Three selected immunoglobulins (IgA, IgG, and IgM) and lactoferrin were chosen to evaluate the effect of microwave-assisted heating and LTLT pasteurization, using ELISA (Table 1). Although ELISA is an effective technique to quantify specific compounds in foods, this assay may not be able to accurately determine the absolute component concentration present in thermally processed foods (Mathiesen et al., 2018). The ELISA assays use specific antibodies to react with specific proteins and any minor modification on the protein structure caused by thermal processes can affect the ability of the antibody to properly recognize the epitopes on a protein, exhibiting a greater degree of underestimation when this method is used to quantify processed foods (Downs & Taylor, 2010). Thus, the specific protein concentrations determined in this study by ELISA can be used to compare the concentrations among the processes to identify treatments that preserved the proteins as their native

**Table 1**

The concentrations of the immunoglobulins (IgA, IgG, IgM) and lactoferrin in donor human milk (DHM), both raw and processed by LTLT pasteurization (62.5 °C for 30 min) and microwave-assisted heating (MWH) at 60 °C for 30 s, 65 °C for 15 s and 70 °C for 10 s.

| Treatments           | IgA (mg/L)              | IgG (mg/L)            | IgM (mg/L)            | Lactoferrin (mg/L)      |
|----------------------|-------------------------|-----------------------|-----------------------|-------------------------|
| DHM raw              | 824 ± 218 <sup>a</sup>  | 51 ± 13 <sup>a</sup>  | 99 ± 7.5 <sup>a</sup> | 2561 ± 557 <sup>a</sup> |
| LTLT pasteurization  | 434 ± 86.4 <sup>b</sup> | 28 ± 1.2 <sup>b</sup> | 32 ± 6.6 <sup>b</sup> | 378 ± 25.8 <sup>b</sup> |
| MWH - 60 °C for 30 s | 677 ± 64.0 <sup>a</sup> | 45 ± 3.6 <sup>a</sup> | 94 ± 9.6 <sup>a</sup> | 2226 ± 234 <sup>a</sup> |
| MWH - 65 °C for 15 s | 684 ± 121 <sup>a</sup>  | 35 ± 3.8 <sup>a</sup> | 88 ± 9.2 <sup>a</sup> | 2186 ± 101 <sup>a</sup> |
| MWH - 70 °C for 10 s | 637 ± 85.7 <sup>a</sup> | 32 ± 2.3 <sup>b</sup> | 50 ± 1.0 <sup>b</sup> | 628 ± 192 <sup>b</sup>  |
| <i>p</i> -value      | 0.044                   | 0.010                 | 0.003                 | 0.002                   |

Means in the same column, with the same letter, did not differ significantly ( $p > 0.05$ ).

structure, but they should not be used to determine the absolute protein concentration in DHM samples.

Among the immunoglobulins studied, IgA had the highest concentration (16-fold and 8-fold higher than IgG and IgM, respectively) in raw DHM and its concentration was not affected ( $p > 0.05$ ) by microwave-assisted heating at all conditions; however, a significantly lower value (47% less) was observed in the IgA concentration after LTLT pasteurization. A similar trend was obtained by Martysiak-Żurowska et al. (2022), which demonstrated that the level of IgA in human milk was not affected after microwave heating at 62.5 °C for 1 min and decreased by 21% after LTLT pasteurization.

The LTLT pasteurization also caused a lower reading for the concentration of IgG, IgM, and lactoferrin, by 45%, 68% and 85%, respectively, whereas the same glycoproteins were not significantly affected by microwave-assisted heating at 60 °C for 30 s. Sousa et al. (2014) also reported lower readings for IgA, IgG and IgM, by 20%, 23% and 51%, respectively, after LTLT pasteurization. Quan et al. (1992) showed no significant differences in IgA concentration after microwave-assisted heating at low temperatures (20–53 °C) for 30 s.



LTLT pasteurization presented the lowest values for original immunoglobulin and lactoferrin concentrations, and this result could be associated with modification of the protein structures, including denaturation, that may have happened during the processing (van Boekel, 1998).

After the thermal treatments, it is important to keep intact the structure of the immunoglobulins and lactoferrin, which have anti-inflammatory (e.g., IgG) and antimicrobial bioactivity, respectively, to regulate the gut flora of infants (Ballard & Morrow, 2013; Zhang et al., 2022). According to Zhang et al. (2022), the risk of necrotizing enterocolitis (NEC) is considerably raised in preterm infants fed with LTLT pasteurized human milk compared with raw human milk, showing the importance of the immunoglobulins and lactoferrin for the lower gut health of the infants. Furthermore, the shorter time required to reach the target temperature and the uniform thermal distribution of microwave-assisted heating probably caused fewer structural protein modifications compared with LTLT pasteurization, thereby not affecting protein concentrations and preserving the beneficial activities of immunoglobulins (Ballard & Morrow, 2013). However, future research needs to be done to investigate the bioavailability of immunoglobulins after microwave heating.

### 3.3. Effect of microwave-assisted heating and LTLT pasteurization on fatty acid concentrations of donor human milk

Based on the data presented above for oligosaccharides, immunoglobulins and lactoferrin, microwave-assisted heating at 60 °C for 30 s was the most efficient pasteurization technique for the preservation of these compounds. Therefore, this condition was chosen to compare the fatty acid concentrations with those found in DHM pasteurized by LTLT (62.5 °C for 30 min) and raw DHM (Table 2).

Nine fatty acids in DHM were identified and categorized as saturated, monounsaturated and polyunsaturated. Fatty acids from the saturated group correspond to 49% of the total fatty acid profile. Gastaldi et al. (2011) also evaluated the fatty acid profile in raw human milk, using the same method as this study, and found a similar saturated fatty acid concentration ( $43.40 \pm 0.94$ ) g/100 g. Yuhas et al. (2006) found a

**Table 2**

Fatty acid concentrations in donor human milk (DHM), both raw and processed by LTLT pasteurization at 62.5 °C for 30 min and microwave-assisted heating (MWH) at 60 °C for 30 s.

| Fatty acid                 | DHM processed by          |                           |                           | p-value |
|----------------------------|---------------------------|---------------------------|---------------------------|---------|
|                            | DHM raw (g/<br>100 g)     | LTLT (g/<br>100 g)        | MWH (g/<br>100 g)         |         |
| Saturated                  |                           |                           |                           |         |
| C10:0 (capric)             | 1.74 ± 0.33 <sup>a</sup>  | 1.82 ± 0.24 <sup>a</sup>  | 1.75 ± 0.35 <sup>a</sup>  | 0.18    |
| C12:0 (lauric)             | 8.01 ± 1.47 <sup>a</sup>  | 8.26 ± 1.60 <sup>a</sup>  | 8.06 ± 1.57 <sup>a</sup>  | 0.89    |
| C14:0 (myristic)           | 8.46 ± 2.26 <sup>a</sup>  | 8.36 ± 2.10 <sup>a</sup>  | 8.47 ± 2.10 <sup>a</sup>  | 1.24    |
| C16:0 (palmitic)           | 24.51 ± 2.59 <sup>a</sup> | 23.83 ± 2.07 <sup>a</sup> | 24.67 ± 2.48 <sup>a</sup> | 1.38    |
| C18:0 (stearic)            | 6.56 ± 1.02 <sup>a</sup>  | 6.51 ± 0.70 <sup>a</sup>  | 7.14 ± 0.78 <sup>a</sup>  | 0.49    |
| Total                      | 49.29 ± 0.91 <sup>a</sup> | 48.78 ± 0.84 <sup>a</sup> | 50.10 ± 0.89 <sup>a</sup> | 1.11    |
| Monounsaturated            |                           |                           |                           |         |
| C16:1 (palmitoleic)        | 1.78 ± 0.26 <sup>a</sup>  | 1.83 ± 0.28 <sup>a</sup>  | 1.74 ± 0.24 <sup>a</sup>  | 0.15    |
| C18:1 n-9 (oleic)          | 27.98 ± 4.34 <sup>a</sup> | 28.08 ± 3.79 <sup>a</sup> | 27.61 ± 4.03 <sup>a</sup> | 2.34    |
| Total                      | 29.76 ± 2.88 <sup>a</sup> | 29.91 ± 2.48 <sup>a</sup> | 29.35 ± 2.68 <sup>a</sup> | 0.32    |
| Polyunsaturated            |                           |                           |                           |         |
| C18:2 n-6 (linoleic)       | 19.55 ± 3.74 <sup>a</sup> | 19.85 ± 3.68 <sup>a</sup> | 19.11 ± 4.08 <sup>a</sup> | 2.22    |
| C18:3 n-3<br>(α-linolenic) | 1.41 ± 0.09 <sup>a</sup>  | 1.47 ± 0.03 <sup>a</sup>  | 1.44 ± 0.10 <sup>a</sup>  | 0.06    |
| Total                      | 20.96 ± 2.58 <sup>a</sup> | 21.32 ± 2.52 <sup>a</sup> | 20.55 ± 2.81 <sup>a</sup> | 1.03    |

Means in the same row, with the same letter, did not differ significantly ( $p > 0.05$ ).

higher proportion of oleic acid in human milk of nursing mothers from China (36 g/100 g) and Canada (35 g/100 g) compared to those from Chile (26 g/100 g) and the Philippines (22 g/100 g). According to the authors, the high content of oleic acid found in the milk of mothers from China and Canada is associated with the high consumption of canola oil by these populations. In this study, an average content of 28 g/100 g of oleic acid was found in raw donor human milk of mothers from Brazil.

Both microwave-assisted heating and LTLT pasteurization did not significantly affect the fatty acid concentrations ( $p > 0.05$ ) in DHM. Henderson et al. (1998) also reported that milk fatty acids, including the polyunsaturated long-chain fatty acids, such as arachidonic acid (C20:4 n6) and docosahexaenoic acid (C22:6 n3), were not affected by LTLT pasteurization; these fatty acids are essential for retinal function and infant brain development. According to Rodríguez-Alcalá et al. (2014), the fatty acid concentrations in human milk are not altered by thermal processing because the aqueous phase of milk acts as an oxygen barrier that hinders oxidation reactions.

No other studies are investigating the effect of microwave-assisted heating on fatty acid concentrations of donor human milk. However, studies using cow's milk as a matrix reported no variation in the fatty acid concentrations after pasteurization and microwave-assisted heating (Cappozzo et al., 2015; Lynch et al., 2005).

### 3.4. Advantages in using microwave-assisted heating to process donor human milk in HMBs

As demonstrated in our previous study (Leite et al., 2019), microwave-assisted heating (60 °C for 30 s) provided the same microbial population reduction as LTLT pasteurization for DHM. In this study, we have further characterized the technique by demonstrating its advantage of preserving immunoglobulins and lactoferrin. Moreover, this microwave technique allows heating at similar temperatures to LTLT but requires a shorter time (30 s, 60-fold shorter than in LTLT), increasing the volume of milk processed in a working day in HMBs. Normally a thermostatic water bath used to pasteurize DHM in HMBs can process an 8-L batch of DHM in 4 h. Moreover, the health professionals, such as nurses, are not generally contracted to exclusive work in HMBs and they share their tasks between the maternity service and the HMB, indicating again the importance of considering the use of faster technology to optimize the working hours of the health professionals.

## 4. Conclusion

In conclusion, the strength of this study is that microwave-assisted heating at 60 °C for 30 s showed promising as an alternative to retain donor human milk main immunological properties related to the presence of immunoglobulins and lactoferrin. With microwave-assisted heating, donor human milk was exposed to a similar temperature but significantly shorter time than LTLT pasteurization, which probably inhibited the degradation and modification of protein structures. Moreover, oligosaccharides and fatty acids were not affected by microwave heating, demonstrating equivalent efficiency with LTLT pasteurization in the preservation of these compounds. In the future, studies investigating the impact of microwave heating on the inactivation of viral foodborne pathogens and preservation of other bioactive compounds, such as vitamins, need to be conducted. Furthermore, in vitro and in vivo studies need to be carried out to better understand the benefits of feeding infants with donor human milk processed by microwave-assisted heating.

## Declaration of interests

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## CRediT authorship contribution statement

**Juliana A.S. Leite:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing. **Randall C. Robinson:** Formal analysis, Investigation, Methodology, Writing – original draft, Writing. **Jaime Salcedo:** Investigation, Methodology. **Juliana N.R. Ract:** Formal analysis, Writing – original draft, Writing. **Virginia S. Quintal:** Formal analysis, Methodology, Resources. **Carmen C. Tadini:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing. **Daniela Barile:** Investigation, Methodology, Resources, Writing – original draft, Writing.

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