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***Staphylococcus aureus* inactivation and maintenance of macronutrients of human milk by high hydrostatic pressure and spray-drying process**

Inactivación de *Staphylococcus aureus* y mantenimiento del valor nutricional de la leche humana por altas presiones hidrostáticas y secado por aspersión

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RESUMEN

Los bancos de leche humana (BLH) utilizan la pasteurización y congelación, como principales métodos de conservación. Sin embargo, su valor nutricional disminuye durante la descongelación y almacenamiento. El objetivo del presente estudio fue evaluar el efecto de las altas presiones hidrostáticas (HHP) y el secado por aspersión sobre los macronutrientes, la calidad microbiológica e inactivación de *Staphylococcus aureus* en LH. Para ello, se realizó la cuantificación de proteínas, lípidos, carbohidratos, cenizas, bacterias lácticas, mesófilos aerobios, coliformes, mohos, levaduras y *S. aureus*. Los resultados mostraron que *S. aureus* y los grupos microbianos evaluados fueron reducidos por debajo del límite permitido por los BLH (<10 UFC/mL). Mientras que las concentraciones de macronutrientes permanecieron sin cambio durante todo el proceso de conservación. El uso de fibra soluble durante el proceso de secado permitió obtener un rendimiento mayor al 99 %. El polvo mostró alta solubilidad y bajos niveles de humedad y actividad en agua; las cuales son propiedades deseables en los alimentos deshidratados. Por lo tanto, la combinación de HHP y el proceso de secado por aspersión demostró ser una alternativa para facilitar el manejo y mejorar la calidad microbiológica de la leche humana.

Palabras clave: Leche humana, altas presiones hidrostáticas, secado por aspersión, *Staphylococcus aureus*, métodos de conservación

ABSTRACT

Human milk banks (HMB) use pasteurization and freezing methods to preserve the milk, nevertheless, the nutritional value of the human milk (HM) decreases during thawing and storage. This study aimed to evaluate the effect of high hydrostatic pressures (HHP) and spray-drying on macronutrients, microbiological quality, and inactivation of *Staphylococcus aureus* on HM. The characterization of HM powder, reduction of *S. aureus*, and modifications in microbiological viability, proteins, carbohydrates, lipids, and ashes were

assessed. The findings demonstrated that while *S. aureus* and all other examined microbial groups were undetectable, macronutrient concentrations remained constant during the entire conservation process. High yield (> 99 %) was achieved thanks to the inclusion of soluble fiber during the drying process, and the produced powder displayed high solubility, low moisture content, and activity water; which are desirable properties in dried foods. Therefore, the combination of HHP and the spray-drying process is an alternative to facilitate handling, improve the microbial quality, allow the addition of oligosaccharides, and maintain the nutritional value of HM in HMB.

Keywords: Human milk, high hydrostatic pressure, spray drying, *Staphylococcus aureus*, milk preservation

INTRODUCTION

Human milk (HM) is a biologically active food that contains high-biological value compounds and immunological constituents, that play a vital role in the modulation of the immune system in newborns and particularly in premature infants. For this, human milk banks (HMB) promote, protect, and support breastfeeding. Besides, these institutions are responsible for collection, conservation, and distribution of milk to feed premature infants and newborns with nutritional disorders (Lowry *et al.*, 1951; Slutzah *et al.*, 2010; Solís-Pacheco *et al.*, 2019). The high availability of nutrients in fresh HM provides an ideal culture media for several microbial groups, such as *Staphylococcus aureus*, one of the most important bacteria that can contaminate HM from origin, due to mastitis by infection (Koenig *et al.*, 2005; Mediano *et al.*, 2017). Besides, secondary contamination can occur due to unsatisfactory hygienic and sanitary handling conditions during extraction and storage (Mediano *et al.*, 2017). To control HM microbial populations, HMB implements several food conservation techniques, with refrigeration and pasteurization as the most widely studied methods (Koenig *et al.*, 2005; Novak and Cordeiro, 2007; Slutzah *et al.*, 2010; Wesolowska *et al.*, 2019). In line with this, HM can be stored at refrigeration temperature

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(4 °C) for up to 96 h, without compromising its nutritional value and microbiological safety (Slutzhah *et al.*, 2010). Instead, pasteurization is the most common technique for food conservation. Microbial inactivation by this process is based on the maintenance of food temperature at 62.5 °C for 30 min and rapid cooling at 5 °C. Pasteurization inactivates vegetative bacteria and most viruses, including human immunodeficiency virus, herpes, and cytomegalovirus (Cavazos-Garduño *et al.*, 2016; Wesolowska *et al.*, 2019). After pasteurization, HM should be stored in airtight containers and frozen at -18 °C for preservation for four months. However, during this period, the nutrients of the milk are not guaranteed (Wesolowska *et al.*, 2019).

Some innovative technologies have been proposed to extend HM shelf-life preserving its nutritional value, including high-temperature short-time pasteurization, high hydrostatic pressure (HHP) processing, and microwave irradiation (Wesolowska *et al.*, 2019). However, once the HM microbial load has been inactivated, HM handling is still complicated due to the high volume required for storage. Recently, the spray-drying process demonstrated to be an alternative for reducing HM volume while maintaining more than 95 % of the macronutrients (Solís-Pacheco *et al.*, 2019). During this process, polysaccharides, proteins, and fiber can be added as wall materials, preventing volatilization and protecting encapsulated material against environmental conditions (Afoakwah *et al.*, 2012). In addition, the use of prebiotic fibers in the HM drying process could act as a source of oligosaccharides for newborns, who cannot obtain them directly from the mother's diet. The value of breastmilk oligosaccharides and dietary fibers, in complementary nutrition for the development of the infant's microbiome with both short- and long-term health complications, has been lately highlighted (Çavdar *et al.*, 2019). NUTRAFLORA® is a synthetic short-chain fructooligosaccharide, made by enzymatic reaction with sucrose. This prebiotic fiber is low in calories (1.5 kcal/g) and viscosity, and has stability at temperatures used during the High-Temperature Short-Time process (> 169 °C). Furthermore, is easy to dry and extrude, and does not participate in Maillard reactions. Its high solubility makes NUTRAFLORA® ideal for dry or liquid formulations (Ingredion, 2016). Hence, this study aimed to evaluate i) the effect of HHP pretreatment and the spray-drying process on the preservation of the macronutrients of HM added with prebiotic fiber, ii) the inhibition of microorganisms naturally present in HM, and iii) the reduction of *S. aureus* artificially inoculated in HM.

MATERIALS AND METHODS

Biological material and ethical considerations

Hospital Civil of Guadalajara "Fray Antonio Alcalde" provide frozen samples from its HMB. The samples were transported in a cooler to the Laboratorio de Microbiología at Instituto Tecnológico de Tepic for analyses. The Ethical Research Committee approved this study on October 2019 No. HCG/CEI-1225/17.

Inoculation of *S. aureus* in HM

S. aureus ATCC 25923 was provided by the Laboratorio de Microbiología Industrial, Universidad de Guadalajara. The stock cultures were kept at -80 °C in tryptic soy broth (TSB; Becton Dickinson Bioxon, Le Pont de Claix, France) with 15 % (v/v) glycerol. Before experiments, 25 µL of stock cultures were transferred to 3 mL TSB and incubated at 35 °C for 18 h, then 25 µL were added to new TSB and incubated at 35 °C under static conditions for 18 h, to yield a final concentration of 10⁶ CFU/mL of microorganisms at stationary phase. Bacterial cells were harvested by centrifugation (9390 × *g* for 5 min), washed twice in sterile saline solution [SS, 0.85 % (w/v), sodium chloride at pH 7.0 ± 0.2], and centrifuged under the same conditions. Then, the bacterial cells were resuspended in the raw HM to a final concentration of 10⁶ CFU/mL (Bulut and Karatzas, 2021).

HM conservation process

For the HHP process, HM was thawed and homogenized. Then 250 mL of non- and inoculated-samples with *S. aureus* were vacuum-sealed in sterile FoodSaver® plastic bags (Newell Brands, Hoboken, NJ, USA) and pressurized at 300 MPa (Avure Autoclave Systems, Model LCIP402260NCEP1MLN, Eri, PA, USA) under different conditions (Table 1). HM was dried using a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland) with an inlet temperature of 165 °C and an air outlet temperature of 110 °C. The milk was fed with a 7 mm-diameter nozzle at a constant flow rate (2 mL/min); during the whole process, the sample was stirred at 100 rpm at 25 °C. Before drying, the spray dryer was stabilized with sterile distilled water (SDW) under the required operating conditions for 10 min. Soluble fiber (NUTRAFLORA® P-95 / L95-S, Ingredion, Ciudad de México, Mexico) was used as wall material at 5 % (w/v). The process yield was estimated (Eq. 1). Three replicates were used for the test and the experiment was repeated twice.

$$\text{Yield (\%)} = \frac{W_{SD}}{W_{SF}} 100 \quad (1)$$

Where: W_{DS} is the weight of dried milk and W_{SF} is the weight of solids in raw milk (Solís-Pacheco *et al.*, 2019). The powder was stored in PET/BOPP/PE zipper bags at 25 ± 2 °C.

Proximal analyses of HM and HM powder

Total protein was estimated by the method of Lowry *et al.* (1951), lipid content was determined by the gravimetric method described by Folch *et al.* (1957), and lactose concentration was obtained with Anthrone reagent following

Table 1. Conditions of high hydrostatic pressure treatments at 300 MPa.

Tabla 1. Condiciones utilizadas en los tratamientos de alta presión hidrostática a 300 MPa.

Treatment	Temperature (°C)	Time (min)
1	35	10
2	35	20
3	45	10
4	45	20

the method of Dubois *et al.* (1956). Protein and lactose quantification were carried out using calibration curves with casein at 595 nm and lactose at 490 nm; respectively. On the other hand, ashes were estimated by drying the samples in an oven at 240 °C for 24 h and the content was expressed as a percentage (Sögüt *et al.*, 2013). Each determination was carried out in triplicate and repeated once. The values were compared with the data obtained from the tests carried out using fresh HM. The methodological strategy realized in this study is represented in Figure 1.

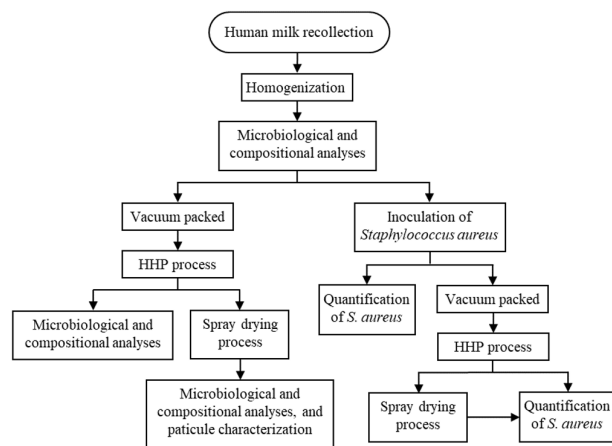


Figure 1. Methodological diagram of the effect of high hydrostatic pressure and spray drying on human milk compositional and microbiological loads.

Figura 1. Diagrama metodológico del efecto de la alta presión hidrostática y el secado por aspersión sobre la composición y carga microbológica de la leche humana.

Microbiological analyses in HM and milk powder

Quantification of mesophilic aerobic bacteria, total coliforms, lactic acid bacteria, *S. aureus*, molds, and yeasts was carried out in raw milk according to Mexican standards (NOM-111-SSA1-1994, NOM-113-SSA1-1994, NOM-115-SSA1-1994, NOM-184-SSA1-2002). Microbial counts were carried out on Petri dishes with the corresponding culture media for each microbial group and incubated under different conditions. For yeast and molds, potato dextrose agar (PDA, Bioxon, Becton Dickinson and Company, Queretaro, Mexico) was used and the plates were incubated for 3 and 5 days at 25 °C. The culture media used for aerobic bacteria (tryptic soy agar, TSA; Becton Dickinson, Le Pont de Claix, France), total coliforms (Red violet bile agar, Merck, KGaA, Darmstadt, Germany), lactic acid bacteria (MRS agar, Oxoid, Basingstoke, UK), and *S. aureus* [Baird-Parker agar base (Difco, MI, USA) supplemented with egg yolk and 100 pg/mL potassium tellurite] were incubated at 37 °C for 24-48 h. Lactic acid bacteria were incubated under anaerobic conditions. Besides, after each conservation process yeast, molds, lactic acid bacteria, and coliforms were estimated as previously described in non-inoculated samples. Whereas, *S. aureus* was quantified in un- and treated inoculated samples. For dried milk, rehydration was carried out considering the moisture content of each sample. The

logarithmic microbial reduction was calculated (Eq. 2).

$$\text{Log}_{10} \left(\frac{N_0}{N} \right) = \text{Log}_{10} N_0 - \text{Log}_{10} N \quad (2)$$

where N_0 is the initial count and N is the survival count after treatment (Coroller *et al.*, 2006). Each test was carried out in triplicate and repeated once.

Moisture content (MC) of spray-dried milk

The MC was gravimetrically determined (da Silva Carvalho *et al.*, 2016). For this, 2 g of sample were used and the thermo-balance (Sartorius MA 35, Göttingen, Germany) was operated at 105 °C, the test was finished until weights remained constant. The test was carried out in triplicate and repeated twice.

Water activity (a_w)

The measurement was made by using 5 g of the sample in the hygroscopic Aqualab 4TEV (METER Group, Inc., Pullman, WA, USA). Calibration was carried out with a sodium chloride solution at 0.75 a_w . The test was carried out in triplicate and repeated twice.

Powder milk solubility

The test was carried out according to the protocol proposed by Cano-Chauca *et al.* (2005). Briefly, 1 g of the powder was added to 100 mL of distilled water and solubilized using a Vortex mixer (Genie 2, Daigger, Wheaton, IL, USA) at 3200 rpm for 5 min. The sample was centrifuged (Hermle Z326K, Wehingen, Germany) for 5 min at 3000 × g , then 25 mL of the supernatant were transferred to pre-weighed Petri dishes and immediately oven-dried at 105 °C for 5 h. The solubility percentage was calculated by weight difference. The test was carried out in triplicate and repeated twice.

Statistical analyses

Factorial designs were carried out. The data were subjected to analysis of variance, then; the post-hoc least significant difference (LSD) Fisher test ($p \leq 0.05$) was used for means comparison. Analyses were performed by using Statgraphics Centurion XVI.I software (Statpoint Technologies, Inc., Warrenton, USA).

RESULTS AND DISCUSSION

Microbiological quality of HM

HM is an ideal medium for the growth of microorganisms due to the high availability of nutrients. Some microorganisms, such as *S. aureus*, *E. coli*, and *Salmonella* may contaminate HM due to inadequate procedures during its extraction and/or storage (Mediano *et al.*, 2017; Novak *et al.*, 2008). In this study, the HM from the Bank of Hospital Civil de Guadalajara showed low levels of aerobic mesophilic bacteria ($2.48 \pm 0.25 \text{ Log}_{10} \text{ CFU/mL}$), *S. aureus* ($2.21 \pm 0.17 \text{ Log}_{10} \text{ CFU/mL}$), and lactic acid bacteria ($1.31 \pm 0.44 \text{ Log}_{10} \text{ CFU/mL}$). Whereas coliforms, molds, and yeast were not detected by the traditional counting plate. The initial counts of all tested microorganisms are below the established limits for pasteu-

rized milk or infant formula ('NOM-131-SSA1-2012, Products and services. Formulas for infants, continuation, and special nutritional needs. Food and non-alcoholic beverages for infants and young children. Provisions and specifications', 2012; 'NOM-184-SSA1-2002, Products, and Services. Milk, Milk Formula, and Milk Product Combined. Sanitary specifications', 2012; 'NOM-243-SSA1-2010, Milk, milk formula, combined milk product, and milk derivatives. Provisions and sanitary specifications. test methods', 2010) considering that the samples were still untreated. These results are in concordance with previous reports of fresh milk obtained from the same HMB (Cavazos-Garduño *et al.*, 2016; Solís-Pacheco *et al.*, 2019; Aguilar-Uscanga *et al.*, 2021). This fact allows us to infer the establishment and compliance of good practices for the obtention and handling of HM by the mentioned HMB. This is of vital importance to maintain microbiological quality and nutritional value, and to avoid infections in newborns via the presence of microorganisms in the milk (Hartmann *et al.*, 2007). However, the microbial load in HM can be higher than that obtained in this research due to some infections, such as mastitis. In HM samples from women with mastitis, the mean microbial load was $4.11 \text{ Log}_{10} \text{ CFU/mL}$. The staphylococcal group is the most frequently isolated (97.57 %), with *Staphylococcus epidermidis* (91.56 %) and *S. aureus* (29.74 %) as the most common species isolated from mastitis samples (Mediano *et al.*, 2017). Inadequate sanitation and handling during milk extraction result in its contamination with *E. coli* and *Salmonella*, while *S. aureus* contamination of HM occurs in breastfeeding women as a result of clinical mastitis (Kaavya *et al.*, 2021; Mediano *et al.*, 2017). In addition, *S. aureus* is present in the oropharynx and skin of humans, and its presence in HM may be due to secondary contamination or to unsatisfactory hygienic and sanitary conditions of the extraction apparatus used (Mediano *et al.*, 2017).

Proximal analysis of HM

The initial estimated values of lactose, lipids, proteins, and ashes in raw HM were 7.36 ± 0.94 , 2.46 ± 0.26 , $1.65 \pm 0.59 \text{ g/dL}$, and $0.26 \pm 0.02 \%$; respectively. These results are in concordance with previous reports for Mexican fresh HM (Cavazos-Garduño *et al.*, 2016; Solís-Pacheco *et al.*, 2019) and with the generally assumed HM composition (Boquien, 2018). After the HHP processing, the assessed components

were maintained at more than 95 % (Table 2). This behavior agrees with the results of Solís-Pacheco *et al.* (2019) for spray-dried HM under the same conditions. However, after the spray-drying process lactose showed a significant reduction in comparison to raw milk ($p \leq 0.05$), but without change in comparison to unpressurized dry HM ($p > 0.05$). During the spray-drying process, chemical and physical changes such as crystallization and nonenzymatic browning as the Maillard reactions can occur, reducing the lactose concentration (Kinsella and Morr, 1984; Uscanga *et al.*, 2021). Besides, the concentration of macronutrients in HM can be influenced by various processes, such as storage, freezing, and thawing. It has been reported that freezing, thawing, and spray-drying processes can decrease the lipid content in HM by up to 9.0 %, attributable to its adherence to the wall of container and equipment, incomplete homogenization, lipolysis, or lipid peroxidation (Cavazos-Garduño *et al.*, 2016; Chang *et al.*, 2012). In this research, the variation in lipid content after HHP and spray-drying were not significant ($p \leq 0.05$; Table 3). The increment in ashes content in the HM powder was attributed to the use of soluble fiber as wall material, which can contain between 1.60 and 2.30 % of ashes depending on the source and variety (Ragaee *et al.*, 2012).

Microbial reduction and inactivation of *S. aureus* by conservation process

Microbial inactivation by HHP depends on several factors including treatment conditions (surrounding media, temperature, pressure, time, etc.) and microbial characteristics (vegetative or spore cells, composition of the cell wall, physiological state, and so on) (Kaavya *et al.*, 2021; Salleh-Mack and Roberts, 2007). The lethal effect of treatments on bacterial cells increased as a temperature function ($p \leq 0.05$; Table 4). This is in agreement with a previous report on the inactivation of *S. aureus* and *Bacillus cereus* spores in HM after 4 cycles of 5 min at 38 °C and 350 MPa (Demazeau *et al.*, 2018). In line with this, it has been demonstrated that *S. aureus* inactivation in cow's whole milk at 20 °C occurs at >250 MPa and 8 min under these conditions, a decimal reduction after 3.7 min at 300 MPa was reported (Erkmen and Karataş, 1997). The composition of the cell wall and shape of the bacteria are related to their resistance. Spherical organisms ought to be more resistant to crushing than those rod-shaped

Table 2. Effect of high hydrostatic pressure on the composition of human milk.
Tabla 2. Efecto de alta presión hidrostática en la composición de la leche humana.

Treatment	Lactose (g/dL)	Lipids (g/dL)	Proteins (g/dL)	Ashes (%)	Humidity (%)
1	6.71 ± 0.69^a	2.89 ± 0.17^a	1.49 ± 0.10^a	0.27 ± 0.03^a	86.19 ± 0.50^a
2	7.42 ± 0.06^a	2.44 ± 0.84^a	1.37 ± 0.64^a	0.20 ± 0.05^a	86.13 ± 0.07^a
3	6.98 ± 0.63^a	1.90 ± 0.70^a	1.44 ± 0.22^a	0.31 ± 0.06^a	86.81 ± 0.68^a
4	6.85 ± 0.60^a	2.34 ± 0.14^a	1.39 ± 0.43^a	0.30 ± 0.09^a	86.37 ± 0.34^a

Values are expressed as mean $\pm$ standard deviation (n = 6). Parameters of raw human milk: lactose $7.36 \pm 0.94 \text{ g/dL}$; lipids $2.46 \pm 0.46 \text{ g/dL}$; proteins $1.65 \pm 0.59 \text{ g/dL}$; and ashes $0.26 \pm 0.02 \%$. Values in the same column followed by different letters are significantly different according to Fisher's LSD test at $p \leq 0.05$.

Table 3. Effect of spray-drying on the composition of human milk.**Tabla 3.** Efecto del secado por aspersión en la composición de la leche humana.

Treatment	Lactose (g/dL)	Lipids (g/dL)	Proteins (g/dL)	Ashes (%)
Control	6.25 ± 0.75 ^{ab}	2.32 ± 0.52 ^a	2.01 ± 0.63 ^a	1.50 ± 0.29 ^{a*}
1	6.99 ± 0.18 ^a	2.23 ± 0.11 ^a	1.83 ± 1.00 ^a	1.32 ± 0.07 ^{a*}
2	5.88 ± 0.39 ^{b*}	2.18 ± 0.41 ^a	1.32 ± 0.82 ^a	1.49 ± 0.11 ^{a*}
3	6.83 ± 0.39 ^a	2.06 ± 0.24 ^a	1.73 ± 0.37 ^a	1.57 ± 0.32 ^{a*}
4	5.74 ± 0.13 ^{b*}	1.95 ± 0.17 ^a	1.30 ± 0.30 ^a	1.34 ± 0.05 ^{a*}

Values are expressed as mean ± standard deviation (n = 6). Parameters of raw human milk: lactose 7.36 ± 0.94 g/dL; lipids 2.46 ± 0.46 g/dL; proteins 1.65 ± 0.59 g/dL; and ashes 0.26 ± 0.02 %. Control: raw or unpressurized dried milk. *: Indicates a significant difference in comparison with the initial value in raw milk. Values in the same column followed by different letters are significantly different according to Fisher's LSD test at $p \leq 0.05$.

Table 4. Reduction of *Staphylococcus aureus* after the conservation process.**Tabla 4.** Reducción de *Staphylococcus aureus* después de los tratamientos de conservación.

Treatment	HHP	HHP + Spray drying
1	2.45 ± 0.53 ^a	ND
2	2.40 ± 0.55 ^a	ND
3	ND	ND
4	ND	ND

HHP: High hydrostatic pressure. Values are expressed as mean ± standard deviation (n = 6). Initial load 6.98 ± 0.23 CFU/mL. Values in the same column followed by different letters are significantly different according to Fisher's LSD test at $p \leq 0.05$. ND: the microorganism was not detected after the treatment.

ones, being consistent with the idea that the lethal action of pressure changes that accompany their passage through liquids (Jacobs and Thornley, 1954). The HHP disrupts the bacterial membrane, being Gram-positive more resistant than Gram-negative bacteria (Costello *et al.*, 2021; Huu *et al.*, 2021; Repine *et al.*, 1981). This is related to the different peptidoglycan content, which is higher in Gram-positive (30-70 %) than in Gram-negative (< 10 %) bacteria, making them more resistant to pressure (Schumann, 2011). Microorganisms can resist high pressures, due to membrane fluidity. However, the synergistic effect of the temperature contributes to the destabilization of the outer and inner membrane, releasing intracellular components (Huu *et al.*, 2021; Kaavya *et al.*, 2021; Salleh-Mack and Roberts, 2007).

Spray drying is a technique widely used for milk powder production. This process is not aimed to cause microbial inactivation

(Alvarenga *et al.*, 2018). However, it showed a synergistic effect in pressurized HM samples contributing to the complete inactivation of *S. aureus* during the drying process, because cells suffered previous damage by the HHP (Bulut and Karatzas, 2021) (Table 4). Temperature can have a cumulative or synergistic effect (depending on the environmental conditions, particularly the media) on the HHP treatments, favoring microbial reduction (Zenker *et al.*, 2003; Zhang *et al.*, 2020). According to Calvoa *et al.* (2018), human milk should be discarded after a pasteurization process, when it has a total microbiological content equal to or greater than 10 CFU/mL, in our case a higher number of bacteria was not found in the human milk samples, after high-pressure processes and spray drying.

Properties of HM powder

The MC of the powders ranged from 2.08 to 2.88 % (Table 5), whereas the microparticles a_w showed values between 0.15 and 0.33. These results agree with the previous report of spray-dried HM without wall materials for which the MC and a_w estimated were 1.7 ± 0.74 % and 0.21 ± 0.15, respectively (Solís-Pacheco *et al.*, 2019). MC and a_w values lower than 5 % and 0.6, respectively, are optimal to reduce microbiological spoilage and prevent lipid oxidation (Sun *et al.*, 2020).

On the other hand, the HM powder solubility was up to 99 %, demonstrating that the macronutrients in HM did not developed insoluble complexes with the fiber, without affecting their availability. This parameter rarely has been assessed for HM, however, is widely studied in dried milk from

Table 5. Properties of dried human milk.**Tabla 5.** Propiedades de la leche humana en polvo.

Treatment	Moisture content (%)	Water activity	Solubility (%)	Yield (%)
Control	2.71 ± 0.79 ^a	0.33 ± 0.14 ^a	99.1 ± 0.04 ^a	97.02 ± 0.13 ^a
1	2.39 ± 0.35 ^a	0.21 ± 0.04 ^a	99.0 ± 0.02 ^a	91.73 ± 1.74 ^b
2	2.88 ± 0.49 ^a	0.20 ± 0.10 ^a	99.0 ± 0.05 ^a	97.02 ± 0.09 ^a
3	2.08 ± 0.38 ^a	0.15 ± 0.05 ^a	99.4 ± 0.02 ^a	88.66 ± 1.98 ^b
4	2.69 ± 0.89 ^a	0.18 ± 0.01 ^a	99.2 ± 0.03 ^a	92.24 ± 2.21 ^b

Control: raw or unpressurized dried milk. Values are expressed as mean ± standard deviation (n = 6). Values in the same column followed by different letters are significantly different according to Fisher's LSD test at $p \leq 0.05$.

other species. For dromedary and cow's milk, the estimated solubility ranged from 91-95 % and 78-88 %; respectively, at an inlet temperature of 230 °C during the drying process (Felfoul *et al.*, 2022). Otherwise, for dried camel milk the solubility was in function of the inlet temperature, being higher at 140 °C than at 200 °C (Habtegebriel *et al.*, 2018). Therefore, the difference between this study and other reports is related to the specie, and the drying processing parameters, due to the milk powder obtained at a less severe processing temperature, the powder retains higher solubility of milk proteins (Habtegebriel *et al.*, 2018).

The yield of the spray-drying process for HM added with soluble fiber was up to 99 %. This result is higher than the obtained for groundnut (82.72 %, inlet temperature 186 °C) (Saha *et al.*, 2019), dromedary and cow milk (94.99 %, inlet temperature 230 °C, and 95.54 %, inlet temperature 230 °C, respectively) (Felfoul *et al.*, 2022). The yield of the spray-drying process is strongly dependent on inlet temperature. Generally is assumed that the use of high inlet temperatures (180-230 °C) provides a higher yield (Felfoul *et al.*, 2022; Saha *et al.*, 2019). However, this is not the only important variable in the yield of dried milk. For camel milk, the total solids recovery in the cyclone increased by an increment of the inlet temperature and airflow rate, whereas decreased with an increase in the total solids content of milk (Habtegebriel *et al.*, 2018). An increment in the total solids of the milk is related to a viscosity increment, difficulty feeding, and aspersion rate resulting in droplets sticking with the drying chamber (Habtegebriel *et al.*, 2018; Langrish *et al.*, 2006). Otherwise, the high yield obtained in this research could be related to the use of soluble fiber, which, additionally, can contribute to macronutrient maintenance. In milk, the concentration of macronutrients in HM can be influenced by various processes, such as storage, freezing, thawing, and spray-drying processes. These techniques can decrease the lipid content in HM by up to 9.0 %, attributable to its adherence to the wall of the dryer chamber and equipment, faults homogenization, lipolysis, or lipid peroxidation (Cavazos-Garduño *et al.*, 2016; Chang *et al.*, 2012). Otherwise, aggregation of casein micelles produces variation in the protein concentration (Chang *et al.*, 2012).

CONCLUSIONS

The combination of HHP and spray-drying technique is an alternative process that allows the maintenance of the value of lactose, lipids, proteins, and ashes in HM. These values were not affected by the use of each treatment alone or in combination. HHP (300 MPa) by itself reduced the microbial loads to undetectable levels, even for *S. aureus* artificially inoculated, if the process was carried out at 45 °C. However, the use of spray-drying after the HHP at 35 °C reduced the remanent microorganisms. Hence, to assure the HM safety the treatment recommendable would be HHP at 45 °C for 10 min followed by the spray-drying process. The transformation of an HM into a powder facilitates its storage and handling in an HMB. The low a_w and MC values are of great importance as

are related to food spoilage reduction in dried foods. Besides, the use of soluble fiber as wall material allowed us to obtain a high yield from the spray-drying process.

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REFERENCES

- Aguilar-Uscanga, B.R., Pacheco, J.R.S., Ragazzo-Sánchez, J.A., Garduño, A.C., Valdéz, J.C.M., Arreola, A. R. and Niño, J.C.S. 2021. Assessment of the accelerated shelf life of human milk dehydrated by aspersion and treated by UV, high pressures, and pasteurization. *Journal of Food Quality*. 2021.
- Afoakwah, A., Adomako, C., Owusu, J., Engman, N., and Hannah, A.A. 2012. Spray drying as an appropriate technology for the food and pharmaceutical industries-a review. *Journal of Environmental Science, Computer Science and Engineering and Technology*. 1: 467-476.
- Alvarenga, V.O., Campgnollo, F.B., Pia, A.K.R., Conceição, D.A., Abud, Y., Sant'Anna, C., Hubinger, M.D. and Sant'Ana, A.S. 2018. Quantifying the responses of three *Bacillus cereus* strains in isothermal conditions and during spray drying of different carrier agents. *Frontiers in Microbiology*. 9: 1113.
- Boquien, C.Y. 2018. Human milk: An ideal food for nutrition of preterm newborn. *Frontiers in Pediatrics*. 6: 295.
- Bulut, S. and Karatzas, K.A.G. 2021. Inactivation of *Escherichia coli* K12 in phosphate buffer saline and orange juice by high hydrostatic pressure processing combined with freezing. *LWT - Food Science and Technology*. 136: 110313.
- Calvo, J., García Lara, N.R., Gormaz, M., Peña, M., Martínez, M.J., Ortiz, P., Brull, J.M. Samaniego, C.M. and Gayà, A. 2018. Recomendaciones para la creación y el funcionamiento de los bancos de leche materna en España. 89(1): Asociación Española de Pediatría. 65.e1-65.e6.
- Cano-Chauca, M., Stringheta, P.C., Ramos, A.M. and Cal-Vidal, J. 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. *Innovative Food Science and Emerging Technologies*. 6: 420-428.
- Cavazos-Garduño, A., Serrano-Niño, J.C., Solís-Pacheco, J.R., Gutierrez-Padilla, J.A., González-Reynoso, O., García, H.S. and Aguilar-Uscanga, B.R. 2016. Effect of pasteurization, freeze-drying and spray drying on the fat globule and lipid profile of human milk. *Journal of Food and Nutrition Research*. 4: 296-302.
- Çavdar, G., Papich, T. and Ryan, E.P. 2019. Microbiome, breastfeeding and public health policy in the United States: The case for dietary fiber. *Nutrition and Metabolic Insights*. 12: 117863881986959.
- Chang, Y.C., Chen, C.H. and Lin, M.C. 2012. The macronutrients in human milk change after storage in various containers. *Pediatrics and Neonatology*. 53: 205-209.
- Coroller, L., Leguerinel, I., Mettler, E., Savy, N. and Mafart, P. 2006. General model, based on two mixed Weibull distributions of bacterial resistance, for describing various shapes of inactivation curves. *Applied and Environmental Microbiology*. 72: 6493-6502.

- Costello, K.M., Velliou, E., Gutierrez-Merino, J., Smet, C., Kadri, H. El, Impe, J.F.V. and Bussemaker, M. 2021. The effect of ultrasound treatment in combination with nisin on the inactivation of *Listeria innocua* and *Escherichia coli*. *Ultrasonics Sonochemistry*. 79: 105776.
- da Silva Carvalho, A.G., da Costa Machado, M.T., da Silva, V.M., Sartoratto, A., Rodrigues, R.A.F. and Hubinger, M.D. 2016. Physical properties and morphology of spray dried microparticles containing anthocyanins of jussara (*Euterpe edulis* Martius) extract. *Powder Technology*. 294: 421-428.
- Demazeau, G., Plumecocq, A., Lehours, P., Martin, P., Couédelo, L. and Billeaud, C. 2018. A new high hydrostatic pressure process to assure the microbial safety of human milk while preserving the biological activity of its main components. *Frontiers in Public Health*. 6: 306.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 28: 350-356.
- Erkmen, O. and Karataş, Ş. 1997. Effect of high hydrostatic pressure on *Staphylococcus aureus* in milk. *Journal of Food Engineering*. 33: 257-262.
- Felfoul, I., Burgain, J., Perroud, C., Gaiani, C., Scher, J., Attia, H. and Petit, J. 2022. Impact of spray-drying conditions on physicochemical properties and rehydration ability of skim dromedary and cow's milk powders. *Drying Technology*. 40: 665-677.
- Folch, J., Lees, M. and Sloane, G.H. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*. 226: 497-509.
- Habtegebriel, H., Edward, D., Wawire, M., Sila, D. and Seifu, E. 2018. Effect of operating parameters on the surface and physico-chemical properties of spray-dried camel milk powders. *Food and Bioproducts Processing*. 112: 137-149.
- Hartmann, B.T., Pang, W.W., Keil, A.D., Hartmann, P.E. and Simmer, K. 2007. Best practice guidelines for the operation of a donor human milk bank in an Australian NICU. *Early Human Development*. 83: 667-673.
- Huu, C.N., Rai, R., Yang, X., Tikekar, R.V. and Nitin, N. 2021. Synergistic inactivation of bacteria based on a combination of low frequency, low-intensity ultrasound and a food grade antioxidant. *Ultrasonics Sonochemistry*. 74: 105567.
- Ingredion. (2016). NUTRAFLORA® P-95 / L95-S Fibra Soluble Prebiótica. [Accessed Dec 08 2022] 2021. Available in: <https://www.ingredion.com/content/dam/ingredion/pdf-downloads/cono-sur/NUTRAFLORA.pdf>
- Jacobs, S.E. and Thornley, M.J. 1954. The lethal action of ultrasonic waves on bacteria suspended in milk and other liquids. *Journal of Applied Bacteriology*. 17: 38-56.
- Kaavya, R., Pandiselvam, R., Abdullah, S., Sruthi, N.U., Jayanath, Y., Ashokkumar, C., Chandra K.A., Kothakota, A. and Ramesh, S.V. 2021. Emerging non-thermal technologies for decontamination of *Salmonella* in food. *Trends in Food Science and Technology*. 112: 400-418.
- Kennedy, J., Blair, I.S., McDowell, D.A. and Bolton, D.J. 2005. An investigation of the thermal inactivation of *Staphylococcus aureus* and the potential for increased thermotolerance as a result of chilled storage. *Journal of Applied Microbiology*. 99: 1229-1235.
- Kinsella, J.E. and Morr, C.V. 1984. Milk proteins: Physicochemical and functional properties. *C R C Critical Reviews in Food Science and Nutrition*. 21: 197-262.
- Koenig, Á., de Albuquerque, E. M., Correia, S. F. and Costa, F. A. 2005. Immunologic factors in human milk: The effects of gestational age and pasteurization. *Journal of Human Lactation*. 21: 439-443.
- Langrish, T.A.G., Marquez, N. and Kota, K. 2006. An investigation and quantitative assessment of particle shape in milk powders from a laboratory-scale spray dryer. *Drying Technology*. 24: 1619-1630.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*. 193: 265-275.
- Mediano, P., Fernández, L., Jiménez, E., Arroyo, R., Espinosa-Martos, I., Rodríguez, J.M. and Marín, M. 2017. Microbial diversity in milk of women with mastitis: Potential role of Coagulase-Negative Staphylococci, Viridans Group Streptococci, and Corynebacteria. *Journal of Human Lactation*. 33: 309-318.
- Norma Oficial Mexicana NOM-111-SSA1-1994, Bienes y servicios. Método para la cuenta de mohos y levaduras en alimentos. [Accessed May 15 2015] 1994. Available in: https://www.dof.gob.mx/nota_detalle.php?codigo=4881226&fecha=13/09/1995#gsc.tab=0
- Norma Oficial Mexicana NOM-113-SSA1-1994, Bienes y servicios. Método para la cuenta de microorganismos coliformes totales en placa. [Accessed May 15 2015] 1994. Available in: <http://www.ordenjuridico.gob.mx/Documentos/Federal/wo69536.pdf>
- Norma Oficial Mexicana NOM-115-SSA1-1994, Bienes y servicios. Método para la determinación de *Staphylococcus aureus* en alimentos 1994. [Accessed May 16 2015] 1994. Available in: <http://www.ordenjuridico.gob.mx/Documentos/Federal/wo69539.pdf>
- Norma Oficial Mexicana NOM-131-SSA1-2012, Productos y servicios. Fórmulas para lactantes, de continuación y para necesidades especiales de nutrición. Alimentos y bebidas no alcohólicas para lactantes y niños de corta edad. Disposiciones y especificaciones 2012. [Accessed May 02 2015] 1994. Available in: https://www.dof.gob.mx/nota_detalle.php?codigo=5267447&fecha=10/09/2012#gsc.tab=0
- Norma Oficial Mexicana NOM-184-SSA1-2002, Productos y servicios. Leche, fórmula láctea y producto lácteo combinado. Especificaciones sanitarias. [Accessed May 04 2015] 2002. Available in: https://dof.gob.mx/nota_to_doc.php?codnota=690308#:~:text=NOM%2D184%2DSSA1%2D2002,23%20de%20octubre%20de%202002
- Norma Oficial Mexicana NOM-243-SSA1-2010, Leche, fórmula láctea, producto lácteo combinado y derivados lácteos. Disposiciones y especificaciones sanitarias. Métodos de prueba. [Accessed April 18 2015] 2010. Available in: <https://dof.gob.mx/normasOficiales/4156/salud2a/salud2a.htm>
- Novak, F.R. and Cordeiro, D.M.B. 2007. The correlation between aerobic mesophilic microorganism counts and Dornic acidity in expressed human breastmilk. *Jornal de Pediatria*. 83: 87-91.
- Novak, F.R., Junqueira, A.R., Dias, M.D.S.P. C. and Almeida, J.A.G. 2008. Sensorial analysis of expressed human milk and its microbial load. *Jornal de Pediatria*. 84: 181-184.
- Ragaei, S., Guzar, I., Abdel-Aal, E.S.M. and Seetharaman, K. 2012. Bioactive components and antioxidant capacity of Ontario hard and soft wheat varieties. *Canadian Journal of Plant Science*. 92: 19-30.

- Repine, J.E., Fox, R.B. and Berger, E.M. 1981. Hydrogen peroxide kills *Staphylococcus aureus* by reacting with staphylococcal iron to form hydroxyl radical. The Journal of Biological Chemistry. 256: 7094-7096.
- Saha, D., Nanda, S.K. and Yadav, D.N. 2019. Optimization of spray drying process parameters for production of groundnut milk powder. Powder Technology. 355: 417-424.
- Salleh-Mack, S. Z. and Roberts, J. S. 2007. Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922. Ultrasonics Sonochemistry. 14: 323-329.
- Schumann, P. 2011. Peptidoglycan structure. In Methods in Microbiology. 38, pp. 101-129. Cambridge: Academic Press, United Kingdom.
- Slutzah, M., Codipilly, C.N., Potak, D., Clark, R.M. and Schanler, R.J. 2010. Refrigerator storage of expressed human milk in the neonatal intensive care unit. Journal of Pediatrics. 156: 26-28.
- Sögüt, Ö., Bali, T., Baltas, H. and Apaydin, G. 2013. Determination of trace elements in ashes of milk samples by using XRF technique. Asian Journal of Chemistry. 25: 4385-4388.
- Solís-Pacheco, J.R., Rodríguez-Arreola, A., Gutiérrez-Padilla, J.A., García Morales, E., Castro-Albarrán, J., Balcázar-López, E., Cavazos-Garduño, A. and Uscanga-Aguilar, B.R. 2019. Human milk powder an alternative for better conservation and healthier use in the banks of human milk. CPQ Nutrition. 3: 1-12.
- Sun, X., Cameron, R.G. and Bai, J. 2020. Effect of spray-drying temperature on physicochemical, antioxidant and antimicrobial properties of pectin/sodium alginate microencapsulated carvacrol. Food Hydrocolloids. 100: 105420.
- Wesolowska, A., Sinkiewicz-Darol, E., Barbarska, O., Bernatowicz-Lojko, U., Borszewska-Kornacka, M.K. and van Goudoever, J.B. 2019. Innovative techniques of processing human milk to preserve key components. Nutrients. 11: 1169.
- Zenker, M., Heinz, V. and Knorr, D. 2003. Application of ultrasound-assisted thermal processing for preservation and quality retention of liquid foods. Journal of food protection. 66: 1642-1649.
- Zhang, H., Tikekar, R.V., Ding, Q., Gilbert, A.R. and Wimsatt, S. T. 2020. Inactivation of foodborne pathogens by the synergistic combinations of food processing technologies and food-grade compounds. Comprehensive Reviews in Food Science and Food Safety. 19: 2110-2138.