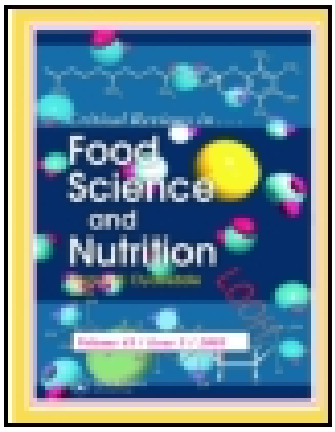

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Human Milk Composition and Preservation: Evaluation of High-Pressure Processing as a Non-Thermal Pasteurisation Technology

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Human Milk Composition and Preservation: Evaluation of High-Pressure Processing as a Non-Thermal Pasteurisation Technology

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Human milk is seen not only as a food, but as a functional and dynamic biologic system. It provides nutrients, bioactive components and immune factors, promoting adequate and healthy growth of newborn infants. When mothers cannot supply their children, donated breast milk is the nutrition recommended by the World Health Organization (WHO), as it is a better alternative than infant formula. However, because of the manner in which donor milk is handled in human milk banks (HMB) many of the properties ascribed to mother's own milk are diminished or destroyed. The major process responsible for these losses is Holder pasteurisation. High-pressure processing (HPP) is a novel non-thermal pasteurisation technology that is being increasingly applied in food industries worldwide, primarily as an alternative to thermal treatment. This is due to its capacity to inactivate microorganisms while preserving both nutritional and bioactive components of foods. This review describes human milk composition and preservation, and critically discusses HMB importance and practices, highlighting HPP as a potential non-thermal pasteurisation technology for human milk preservation. HPP technology is described and the few currently existing studies of its effects in human milk are presented.

Keywords: human milk composition, milk banks, high-pressure processing, pasteurization

Introduction

During the last decades multiple scientific data have confirmed that breastfeeding is the optimum nutrition for term and preterm infants, at least for the first six months and, if possible, for the first year of life or even more (Bertino et al., 2009). The World Health Organization (WHO) and The United Nations Children's Fund (UNICEF) recommend exclusive breastfeeding for the first six months of life and the introduction of complementary foods at six months together with continued breastfeeding up to two years and beyond (World Health Organization, 2010). Human milk provides several benefits to the infants, including enhancement of host defences, neurological development and gastrointestinal function (Heiman and Schanler, 2007; Lawrence and Pane, 2007). In addition, it enhances antioxidant defences against hydroxyl radical aggression in preterm infants (Ledo et al., 2009).

Thus, besides the role that breastfeeding plays in the normal development of the infant, it also protects against infectious diseases in infancy and childhood. The protective effect of breast milk against diarrhea, respiratory and urinary tract infections, necrotising enterocolitis (NEC), nosocomial sepsis, otitis media and common infections in premature infants has been documented (El-Mohandes et al., 1997; Lawrence and Pane, 2007; Marild et al., 2004; McGuire and Anthony, 2003). Early prospective studies reported lower rates of infection in premature infants being fed fresh human milk when compared with formula milk (Narayanan et al., 1980; Narayanan et al., 1984). In the case of NEC, a published meta-analysis revealed that infants receiving human milk were three times less likely to develop NEC than infants receiving formula milk (McGuire and Anthony, 2003). Another systematic review and meta-analysis suggests that

human milk might reduce the risk of NEC by about 79% when compared to formula milk (Boyd et al., 2007). Breastfeeding has also proven to have a protective effect against obesity in children (Arenz et al., 2004).

Human milk composition

Human milk is currently seen not only as a food, but also as a functional and dynamic biologic system. This complex fluid simultaneously provides nutrients, bioactive components and immune factors like immunoglobulins (Igs), lactoferrin and lysozyme (Field, 2005; Lopez Alvarez, 2007). The composition of human breast milk is not homogeneous; it varies over the course of lactation and between lactating women. It is known that many factors, such as weeks of lactation, breastfeeding time, gestational age, genetic factors and dietary habits are responsible for the variations in human milk components between individuals (Maas et al., 1998; Wojcik et al., 2009). In a single individual, breast milk composition may even change over the course of the day (Shubat et al., 1989) and significantly from day to day (Butte et al., 1988). Therefore, it becomes quite difficult to define a general composition of human milk.

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) (Sala-Vila et al., 2005). The changes in composition are greatest and occur most rapidly during the first week after birth (Emmett and Rogers, 1997). Table 1 shows the basic chemical composition of human milk from healthy women who delivered term infants in the United Kingdom (UK; data from the UK's standard food analysis tables) (Emmett and Rogers, 1997) and Inner Mongolia (China) (Shi et al., 2011). This composition is presented for the three stages of lactation. The

nutrient concentrations of mature milk are in agreement with other authors (Yamawaki et al., 2005) and The United States Department of Agriculture (USDA) reference values (USDA, 2009).

Proteins

Protein and total nitrogen contents are higher in colostrum than in transitional and mature milk (see Table 1). Milk proteins are of two major types: casein and whey proteins, and, unlike cow's milk, approximately two-thirds of the proteins in human milk are whey proteins. The most representative human milk whey proteins are presented in Table 2. Among them the most abundant are α-lactalbumin, lactoferrin and secretory immunoglobulin A (sIgA) (Nagasawa et al., 1973). The higher protein concentration in colostrum is mainly due to the higher concentration of sIgA, but the concentrations of IgM and IgG are also higher in colostral milk than in transitional and mature milk (Table 2). The concentrations of α-lactalbumin and lactoferrin both in colostrum and transitional milk are higher than in mature milk (Shi et al., 2011).

Some proteins of human milk, such as α-lactalbumin, lactoferrin and casein, are synthesised by the mammary gland whereas others, such as serum albumin, are derived from the mother's blood (Lönnerdal et al., 1976). The concentration of those secreted by the mammary gland decreases during the first days of lactation, while those derived from the mother's blood remain fairly constant (Emmett and Rogers, 1997). Igs, lactoferrin and α-lactalbumin, as well as other proteins with antimicrobial activity (e.g. lysozyme and lactoperoxidase), are relatively resistant to proteolysis in the gastrointestinal tract and contribute to the defence of breastfed

infants against pathogenic bacteria and viruses (Lönnerdal, 2003). Infant formulas do not contain this range of antimicrobial proteins. In agreement with this, breastfed infants have shown higher levels of sIgA in saliva than bottle-fed infants (Uruakpa et al., 2002).

IgA, lysozyme and lactoferrin, for example, have a remarkable antimicrobial activity. IgA functions essentially by directly binding to specific microbial antigens, lysozyme acts by degrading the outer cell wall of Gram-positive bacteria, causing bacterial cell wall lysis, and lactoferrin functions via iron chelation, limiting siderophilic bacterial growth (Lawrence and Pane, 2007). Moreover, it has been suggested that lactoferrin has a great potential in cancer disease prevention, protecting against cancer development and metastasis (Rodrigues et al., 2009).

Non-protein nitrogen (NPN) concentration does not vary significantly throughout lactation (Table 1), because NPN is derived mainly from the mother's blood (Lönnerdal et al., 1976). Functional roles of some NPN containing compounds, such as taurine, L-carnitine, free amino acids and free nucleotides are still not clear. Hitherto it is thought that their biological significance is related to the infant growth and development (Ferreira, 2003). Free amino acids are present in human milk in higher number and concentration than in cows' milk, supporting this hypothesis.

Lipids

Fat is the main source of energy in human milk and it is distinct in composition from that in the milk of other animals and generally better absorbed by the infant's gut (Emmett and Rogers, 1997). In what concerns lipid content of human milk the results available in literature are vary

variable. Emmett and Rogers (1997) observed that the total fat content of breast milk in UK women increases from colostrum to transitional and mature milk, while recently Shi et al. (2011) found no significant difference in the total fat between the milk of Inner Mongolia women in the three lactation stages (Table 1). This can happen due to several factors, as fat content of breast milk varies between feeds, depending on the extent to which the breast was emptied during the previous feed, and along a single feed. Lipids appear to be the most variable macronutrient inter- and intra-individuals and with maternal nutrition (Emmett and Rogers, 1997). Regarding the effect of maternal nutrition, it was demonstrated that weight gain during pregnancy was directly related to higher fat concentration in breast milk (Michaelsen et al., 1994). Furthermore, the pattern of fatty acids in maternal diet influences fatty acid composition of milk (Emmett and Rogers, 1997). Table 3 shows the fatty acid composition of English and Chinese women's milk along lactation.

Fatty acids are the main components of milk fat and, while Shi et al. (2011) found no significant variation of these compounds during the three lactation stages, Emmett and Rogers (1997) reported an increasing tendency along the lactation period, mainly for saturated and monounsaturated fatty acids. These differences are probably explained by the intra- and inter-individual fat variability described above. In the case of saturated fatty acids, for example, some authors (Silva et al., 2005) obtained similar results to those found by Shi et al. (2011), whereas others obtained higher concentration values (Sala-Vila et al., 2005).

Among other fatty acids, human milk provides all of the dietary essential fatty acids, such as linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3), as well as other longer-chain more-unsaturated ones, including arachidonic acid (20:4n-6) and docosahexaenoic acid (DHA;

22:6n-3). These fatty acids support the growth and development of breastfed infants (Innis, 2007b). Shi et al. (2011) found a ratio of LA to ALA of 5.2:1 which is in the range of 5:1 to 15:1 recommended by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) committee on nutrition. However, these authors did not detect arachidonic acid and DHA in the milk of Chinese women, which were detected by other authors in Brazilian and Spanish mothers (Sala-Vila et al., 2005; Silva et al., 2005). Since LA and ALA cannot be formed by mammalian cells, their presence in milk results from the maternal diet (Innis, 2007b).

DHA is of great importance, because it is accumulated specifically in the membrane lipids of the brain and retina, where it plays critical roles in the visual and neural systems. These roles include protection from oxidative damage, neurogenesis, neurotransmitter metabolism, membrane protein functions, transmission of the visual signal and regulation of gene expression (Innis, 2007a). Archidonic acid and DHA are not essential in adult diets, but preterm and very young infants cannot synthesise them fast enough to keep up with their nutritional needs (Emmett and Rogers, 1997). Most infant formulae do not contain the whole range of fatty acids present in human milk and are low in some, particularly in long chain poly-unsaturated fatty acids (PUFAs) (Emmett and Rogers, 1997).

Carbohydrates

The concentrations of total carbohydrates and lactose do not vary significantly between colostral, transitional and mature milk (Table 1). Lactose is the main carbohydrate in human milk (≈ 7 g/100 mL) and its concentration seems to be fairly insensitive to changes in diet and nutritional

status (Emmett and Rogers, 1997). In newborns lactose is not entirely hydrolysed in the intestine and the small amounts of unhydrolysed lactose that reach the colon are consumed by bacteria, resulting in the preferential growth of bifidobacteria and lactobacilli (Coppa et al., 2006). Besides lactose, oligosaccharides are also present in substantial amounts in colostrum (2.1 g/100 mL on day 4) and mature milk (1.3 g/100 mL on day 120) (Coppa et al., 1993). Oligosaccharides, together with proteins, are the third major components of human milk from a quantitative point of view, right after lactose and lipids (Zivkovic et al., 2011).

The prebiotic role of oligosaccharides has been thoroughly described in literature and it is currently undoubted that they promote a bifidobacteria-dominant microflora, contributing to the healthy growth of infants. Several studies outline that they resist digestion and reach the colon where they stimulate the development of the bifidus-predominant flora (Coppa et al., 2006). Only trace amounts of oligosaccharides are present in cows' mature milk (Lane et al., 2010) and, consequently, in cow's milk-based infant formula. Hence, breastfed infants' microflora shows a predominance of bifidobacteria and lactobacilli (90%) (Harmsen et al., 2000), while formula-fed infants' intestinal flora has a significantly lower number of these bacteria (40-60%), with the remaining composed of *Enterobacteriaceae* and *Bacterioides* (Harmsen et al., 2000; Rubaltelli et al., 1998). In a lesser amount, monosaccharides, mostly glucose and fuccose, are present too (Emmett and Rogers, 1997).

Vitamins

Maternal milk should fulfil all of the vitamins requirements of newborns, but vitamin content may vary significantly according to the maternal diet and, specifically, to vitamin dietary intake.

Table 4 presents the human milk vitamins composition of women who gave birth in the UK and in China, two countries with different dietary habits.

Water-soluble vitamins concentrations are usually more affected by maternal diet than the concentrations of fat-soluble vitamins (Prentice et al., 1983). Emmett and Rogers (1997) reported that soluble vitamins as thiamine, niacin, vitamin B_6, folate, pantothenate and biotin increase in concentration from colostrum to mature milk, while vitamin B_{12} and vitamin C decrease and riboflavin concentration remains the same. However, even though the similarity of concentrations, Shi et al. (2011) found that water-soluble vitamins, such as thiamin, niacin, vitamin B_6, folate, pantothenate, riboflavin and vitamin C did not vary significantly along lactation. These differences may be explained by variations in the maternal diet. Also, the concentration of vitamin C found by Shi et al. (2011) throughout lactation was lower than the concentration reported by Emmett and Rogers (1997). This can be due to traditional Chinese dietary habits of many mothers that exclude most fruits and vegetables from their diet during 15 to 30 days after parturition, leading to a great decrease of vitamin C (Shi et al., 2011). In general, the results of both works were similar to the USDA reference values (USDA, 2009).

Among fat-soluble vitamins, those that are most relevant in human milk are vitamin A or retinol and vitamins D, K and E. Emmett and Rogers (1997) observed a decrease in the content of vitamins A and E of human milk over the course of lactation. On the other hand, Shi et al. (2011) found a major decrease of vitamins D and K from colostrum to mature human milk. Vitamin A concentration varies according to the mother's diet in pregnancy and lactation, that is, according to the vitamin A status of the mother (Mello-Neto et al., 2009). Thus, lower retinol concentrations found in Chinese women may be associated with maternal food choice in this

region (more eggs and rice) (Shi et al., 2011). Regarding vitamins D and K, human milk contains small amounts of these vitamins. Nevertheless, they are important in the prevention of some diseases (Ala-Houhala, 1985; Lane and Hathaway, 1985). Although vitamin E concentration in Chinese mothers was lower than reference values (Emmett and Rogers, 1997; USDA, 2009), there is little evidence of a relation between the mother's diet and vitamin E concentration in breast milk (Emmett and Rogers, 1997).

Minerals

The bioavailability of most minerals in human milk is much higher than in cow's milk or infant formula (Emmett and Rogers, 1997), so that they can be efficiently used by the organism as they are present in milk in low concentrations. Results from Emmett and Rogers (1997) and Shi et al. (2011) are presented in Table 5 and show similar concentrations of calcium (Ca), phosphorous (P), chlorine (Cl; except for mature milk), magnesium (Mg), cooper (Cu), iron (Fe), zinc (Zn; except for colostrum) and selenium (Se). However, Shi et al. (2011) obtained lower concentrations of potassium (K) and sodium (Na) and higher concentrations of iodine (I), than those presented by Emmett and Rogers (1997). Other studies that measured mineral and trace element composition of breast milk (Almeida et al., 2008; Yamawaki et al., 2005) showed similar concentrations to those obtained in the works used to create Table 5 (Emmett and Rogers, 1997; Shi et al., 2011).

The observed differences are probably due to factors included in the intra- and inter-individual variation, such as maternal diet or nutritional status. The effect of maternal diet in the mineral content of human milk is not the same for all minerals, yet depends on which mineral is

being considered. Maternal intake of certain minerals like calcium, magnesium, cooper and zinc does not seem to affect the breast milk concentration (Aggett, 1994; Garg et al., 1988). On the other hand, there is evidence that selenium concentrations in breast milk can be affected by maternal diet (Funk et al., 1990).

Human milk banks

Human milk banks (HMB) are essential to nourish both term and preterm infants who cannot be breastfed (or at least exclusively) (Morales and Schanler, 2007), as they provide donor milk, which is a better alternative than infant formula (Leaf and Winterson, 2009). Even artificial formula manufacturers have already acknowledged that "Formula or cow's milk is low in functional components and can never be fortified to match breast milk" (Yeung and Peters, 2001). In this sense, the enormous volumes of human milk discarded by mothers who produce more than their infants require is an invaluable resource if donated to HMB (Hartmann et al., 2007).

Human milk banking consists in collecting, processing and storing human milk from lactating women with the aim of feeding it to other women's babies. This is not a new concept; wet-nursing has been carried out for centuries and since 1909, when the first milk bank appeared, milk banking has been practised all around the world (Leaf and Winterson, 2009). Later on in the 1900's the development and assertive marketing of bovine infant formulas, as well as the emergence of HIV and the fear of other viruses and illness, hospital budget crisis and progress in the neonatal intensive care changed this situation, leading to closings of many banks (Jones, 2003; Leaf and Winterson, 2009). Nowadays, with increasing awareness of donor human milk

benefits and safety, the interest in HMB is resurging along with the required resources (Tully et al., 2004).

HMB are expanding in number and capacity and are particularly focused in providing human milk to extremely premature (gestational age below 28 weeks), premature (28 – 33 weeks) or ill hospitalised infants, as they need for larger amounts of protein and energy than the healthy term ones to achieve appropriate growth (Sauer, 2007). Thus, whenever possible, the milk from mothers who give birth to extremely premature or premature infants (extremely preterm and preterm milk, respectively) is preferred, because of its higher contents of protein, fat, carbohydrates and energy (Bauer and Gerss, 2011). Nevertheless, processing and storage of donor milk, as well as mothers' own milk, affect some of its nutritional and immunological properties (Wight, 2001).

The macronutrient composition of pasteurised donor human milk, mainly from mothers who gave birth prematurely, was analysed in the Perron Rotary Express Milk Bank in Australia (Hartmann et al., 2007). Protein, lactose and energy concentrations were similar to those of unpasteurised mature human milk from healthy mothers of term infants reported by other authors (Emmett and Rogers, 1997; Shi et al., 2011). Fat concentration was slightly higher than that obtained by Shi et al. (2011) and similar to that reported by Emmett and Rogers (1997). As above referred, it is known that preterm milk has higher macronutrient content than term milk. Hence the similar macronutrient concentrations between pasteurised preterm milk and raw term milk are probably due to losses during human milk pasteurisation.

Figure 1 represents the WHO hierarchy of feeding choices for low-birth-weight infants (Arnold, 2002), which is very similar to that for regular weight babies except for the requirement

of preterm milk. Breastfeeding is always the number one option, but for premature or sick infants whose mothers cannot breastfeed, donated fresh preterm milk becomes the best option and donated fresh term mature milk the second best. Pasteurised donated breast milk is the number four option in WHO choices, because pasteurisation diminishes the nutritional value of human milk and partially destroys immunological and functional factors. This is described further in section "Effects of pasteurisation, freezing/thawing and storage". Preterm and ordinary formula come only as the last choices (numbers five and six, respectively).

Human milk banking practices

The number of HMB is increasing day by day. Therefore, in the absence of international regulations and worldwide recognised guidelines, several countries have developed their own guidelines, procedures, and quality standards (Baumer, 2004; Dall'Oglio et al., 2009; Hartmann et al., 2007; Melo et al., 2010; Updegrove, 2005). In UK for example, HMB can voluntarily adopt the standards of practice of the UK Association of Milk Banks (www.ukamb.org), which are different from those of the Human Milk Banking Association of North America (www.hmbana.org), followed in Canada, Mexico and the United States of America (USA) (Modi, 2006). There are few countries, such as Brazil, where governments have specific legislation regulating milk banking and difficulties seem to be greatly reduced in this way (Arnold, 2006). The general milk banking practices will be described and explained, taking as example the milk banks of the Human Milk Banking Association of North America (HMBANA), as some procedures differ between countries. In Figure 2 a simplified scheme of the main practices performed in HMB in order to collect and preserve milk is presented.

The whole process starts with the choice of the donors. Donors are self-selected in the first place and are not paid, their satisfaction comes from the fact that they are able to help other babies in need (Updegrove, 2005). After a potential donor manifests the interest in donating her milk the donor screening begins. Firstly, mothers are questioned about mother and baby's health, maternal lifestyle and medical issues. Secondly, they are informed about the process of pumping and storing milk and asked to do some blood tests (HIV-1, HIV-2, human T cell lymphotropic virus (HTLV), hepatitis B and C, and syphilis) (Hartmann et al., 2007).

After the blood tests results are known the donors may be approved, denied or asked to answer some more questions, so that a decision can be made (Updegrove, 2005). HMBANA donor exclusion criteria are quite extensive and include any positive blood test among the required ones, risk factors for HIV, use of illegal drugs, use of nicotine products, regular alcohol intake, receiving a blood transfusion in the last 4 months or an organ or tissue transplant in the last 12 months, and travelling to the UK for more than 3 months (1980-1996) or Europe for more than 5 years since 1980 (HMBANA, 2012). The last criterion is presumably based on the risks of Creutzfeldt-Jakob disease (Modi, 2006) and obviously is not applied in European countries.

Once the donor is approved she has to express the milk, manually or through the use of a breast pump, collect it to disinfected polypropylene bottles, label them and freeze the milk in the coldest part of the freezer (Hartmann et al., 2007). Then, she has to deliver it to the milk bank or other existing collection sites in the area (Updegrove, 2005). In the milk bank, raw donor human milk is stored frozen at – 20 ºC to prevent microbial growth (Pardou et al., 1994) and lipid peroxidation, to reduce viable cytomegalovirus (CMV) and preserve vitamin C content (Baumer, 2004). Before being delivered to medically needy individuals or medical institutions, the milk

has to be mandatorily pasteurised to inactivate vegetative pathogenic microorganisms, part of the commensal flora (Molto-Puigmarti et al., 2011) and also viruses. In accordance with the guidelines of the National Institute for Health and Clinical Excellence (NICE) for the operation of milk bank services, donor milk awaiting pasteurisation in the freezer should be kept there for no longer than 3 months after expression (NICE, 2010). Currently, the most frequently used method is in-pack pasteurisation, known in the field as Holder pasteurisation, which is a low-temperature long-time (LTLT) pasteurisation technique. It consists in placing the packaged milk in a water bath and heating it at 62.5 ºC for 30 minutes (Updegrove, 2005).

In order to be pasteurised, the milk is thawed, poured into flasks and mixed carefully to promote homogenisation. Then, a sample of raw milk is taken for microbial quality control. Human milk that tests positive for certain bacteria as *Staphylococcus aureus* or any of the bacillus species is discarded. This is due to the fact that germination of spores of bacillus species, for example *Bacillus cereus*, can occur during heat treatment (Hanson et al., 2005). After pasteurisation a second sample is taken for microbiological control. Any bacterial growth in the post-pasteurised sample is unacceptable and leads to the discarding of that donor's milk (Updegrove, 2005). Usually, another sample is collected from the donor's milk for nutritional analysis, namely energy, fat, protein and lactose contents. This happens before or/and after pasteurisation, depending on HMB individual procedures.

In the Mother's Milk Bank at Austin (MMBA) one sample is collected before pasteurisation with the aim of evaluating the nutritional value of human milk and another one after to recheck the nutritional content (Updegrove, 2005). Based on the obtained results, milk from two or three donors is mixed together, in order to ensure an adequate caloric and protein

value for the milk; given that human milk composition is very variable. At the end the bottles are labelled, which include nutritional information, and the milk is pasteurised. Following pasteurisation the milk is quickly cooled in an ice bath and frozen at – 20 ºC for dispensing (Updegrove, 2005). Frozen pasteurised donor milk is typically stored for no longer than 6 months after the date of expression (NICE, 2010).

There are, however, some exceptions to these general procedures. Milk banks in the UK, for example, do not pool donor milk, yet they prepare it in aliquots from individual donors to minimise the risk of multiple donor exposure (Leaf and Winterson, 2009). In some countries like Norway, HMB do not pasteurise donor milk; instead they use raw donor milk to feed preterm infants (Grøvslien and Grønn, 2009). The donors have to be screened for HIV, hepatitis B and C, HTLV and CMV and the milk screened for bacteria to ensure that it is free of pathogens and has low bacterial counts. Also in 5 of the 27 Swedish HMB raw donor milk is used (Omarsdottir et al., 2008).

One of the main constraints of HMB is their financial implications and the high costs of the final product. Even though donors of human milk are not paid, the costs of screening, pasteurisation, storage and transportation are high. Thereby, some authors have made simple comparisons in order to assess economical potential of these banks. As recently reviewed by Leaf and Winterson (2009) in UK, costs of transport, pasteurisation, storage, staff training and administration were determined to be up to £150/litre (Tully, 2000). It is much higher than the cost of preterm formula (around £10/litre), but of minor significance when compared to the cost of a neonatal bed (around £189-£355/day in 2000) (O'Neill et al., 2000).

Likewise, Simmer and Hartmann (2009) compared the cost of pasteurised donor human milk with the cost of staying in the neonatal intensive care unit (NICU). In the USA, private non-profit milk banks charged about US$3.00 per 30 mL of donor human milk in 2009, which was equivalent to AU$120 per litre at the time. Following Australian NICU standards, it was estimated that a hypothetical infant being fed exclusively donor milk for 24 weeks would require 10 L of milk, which would cost around AU$1200. This cost is equivalent to less than a day's care in their NICU. Therefore, if providing donor milk helps prevent some of the complications attributed to artificial formula use, the length of stay in the NICU may be significantly reduced and the investment greatly monetised (Simmer and Hartmann, 2009).

Effects of pasteurisation, freezing/thawing and storage

Due to the effects of heating, cooling, freezing, and storage, some of the most valued components of human milk are diminished or destroyed. On the other hand, feeding fresh milk (or at least fresh frozen and not heated) preserves most of the components (Lawrence and Lawrence, 2011).

Even though pasteurisation of donor human milk eliminates the risk of transmission of infectious agents, it does affect some of the nutritional and immunological components of human milk (Wight, 2001), as evidenced by several studies. A recent study, which determined the effect of Holder pasteurisation, freezing at – 20 ºC and thawing on fat, protein and lactose concentrations in human milk, found a significant reduction of fat (5.5%) and protein (3.9%) concentrations following these processes (Vieira et al., 2011). It was demonstrated that the major process responsible for these losses was pasteurisation. Lactose concentration did not suffer a

significant reduction (0.5%) throughout the processes studied. A previous study where pooled human milk was subjected to Holder pasteurisation and storage at – 20 ºC up to 90 days obtained similar reduction of fats (of 6%) (Lepri et al., 1997). In addition, both of the works are in agreement that freezing and thawing may alter the structure of the fat globule, causing lipolysis of breast milk.

Lepri et al. (1997) also reported that pasteurisation induced triglyceride hydrolysis. In this work, the amount of free fatty acids (FFAs) doubled after pasteurisation and rose even more after storage. Wardell et al. (1981) found lower percentage of linoleic (C18:2n-6; -22%) and linolenic (C18:3n-3) acids in milk after freezing, thawing and Holder pasteurisation. Another study found that the proportion of medium-chain saturated fats (C12:0, C14:0) was increased after pasteurisation, with a trend towards a decreased proportion of oleic acid (C18:1n-9) (Ewaschuk et al., 2011). In contrast, other studies showed no significant differences in human milk fatty acid proportions before and after pasteurisation (Fidler et al., 1998; Henderson et al., 1998; Romeu-Nadal et al., 2008). Nevertheless, a randomised study found that feeding preterm infants with pasteurised milk as compared with raw milk reduced fat absorption in 17%, causing them to gain less weight (Andersson et al., 2007). There is the likelihood that these last results are due to inactivation of milk lipases during pasteurisation, which will be discussed further on, and the consequential removal of the filaments from milk fat globules (Buchheim et al., 1988). Besides these macronutrients, it has been demonstrated that Holder pasteurisation does not affect the concentration and pattern of oligosaccharides in human milk (Bertino et al., 2008).

The most severe effects of Holder pasteurisation on human milk are reported to be on immune factors and certain enzymes. As reviewed by Heiman and Schanler (2007), the main

immune factors and enzymes decrements are: sIgA (20 to 50%), total IgA (0 to 50%), lactoferrin (0 to 65%), lysozyme (0 to 65%), lymphocytes (100%), lipase (100%) and alkaline phosphatase (100%) (Baum, 1980; Garza et al., 1986; Hamprecht et al., 2004; Koenig et al., 2005). In addition, it is estimated that IgG (Evans et al., 1978) and IgM (Ford et al., 1977) are decreased in 34 and 100%, respectively, by pasteurisation of human milk. Henderson et al. (1998) confirmed that the two lipases present in human milk, lipoprotein lipase (LPL) and bile salt-stimulated lipase (BSSL), were completely inactivated during pasteurisation, while amylase lost about 15% of its initial activity.

Recently, Holder pasteurisation method was shown to structurally modify and decrease BSSL, lactoferrin and components of the immune system, and increase lysine bioavailability (Baro et al., 2011). It was also demonstrated that pasteurisation significantly reduces the concentrations of several immunoactive compounds present in the human milk, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-10 and hepatocyte growth factor (HGF) (Ewaschuk et al., 2011). These results are in accordance with a previous study where the authors observed that cytokine concentrations declined following pasteurisation, and suggested that more pro-inflammatory than anti-inflammatory cytokines are retained (Giorgi et al., 2006). Transforming growth factors α (TGF-α) and β_2 (TGF-β_2) are preserved in milk heated at 56.5 ºC for 30 minutes (McPherson and Wagner, 2001).

Vitamin content before and after Holder pasteurisation was investigated as well, and, while vitamins A, D and E were not affected, the concentrations of vitamins C, folacin and B_6 were lowered in 36, 31 and 15%, respectively (Van Zoeren-Grobben et al., 1987). A latest study confirmed that vitamin E did not vary and that pasteurisation significantly decreased vitamin C

(20%) and ascorbic acid (16%) levels. (Molto-Puigmarti et al., 2011). There were no changes in the mineral content of milk regarding minerals like Ca, P or Na (Williamson et al., 1978).

Cold storage, freezing and thawing of donor human milk are similar to the practices used by a mother with her own milk for her baby, but these processes may affect human milk composition, too. Garza et al. (1986) determined the effects of freezing at – 20 ºC for 3 months in some components of raw human milk. They observed a small decrease in total IgA (3%), but no changes in sIgA or lactoferrin concentrations. Lysozyme concentration was reduced up to 20% and white blood cells were almost completely destroyed. In addition, they verified that storage in the refrigerator at 4 ºC for 24 hours results in several losses: vitamin C (40%), lysozyme (40%), lactoferrin (30%), lipase activity (25%), sIgA (40%), and specific sIgA antibody (0 to 60%). Refrigerated milk showed a reduction of 40% in its phagocytic activity, an increase in the number of cellular elements and also a large increase in FFAs, suggesting spoilage (Garza et al., 1986).

Evans et al. (1978) reported no appreciable losses of lactoferrin, lysozyme, IgA and IgG in human milk after storage by deep freezing at – 20 ºC for 3 months. In agreement with this, Reynolds et al. (1982) found no changes in the levels of IgA, IgM, IgF, lactoferrin, lysozyme, C3 and C4, amino acids and fatty acids after 1 month of storage at – 20 ºC. In contrast, Akinbi et al. (2010) observed significant decreases in lysozyme (32%), sIgA (51%) and lactoperoxidase (66%) after freezing at – 20 ºC during 4 weeks. Recently, Ramírez-Santana et al. (2012) reported the retention of colostrum IgA, epidermal growth factor (EGF), TGF-β1, TGF-β2, IL-6, IL-8, IL-10, TNF-α, and its type I receptor TNF-RI after 6 months of freezing storage at – 20 ºC and – 80 ºC. Nevertheless, after 12 months storage at both temperatures significant losses of IgA, IL-8 and

TGF-β1 were verified. Moreover, cold storage at 4 ºC for 48 h resulted in the maintenance of all of these bioactive immunologic factors, excepting for IL-10.

It can be concluded that collecting, processing, and storing human milk substantially diminishes its most valued properties. This decrease in human milk functionality and quality is mainly due to the pasteurisation heat treatment that is the bottleneck to obtain microbiologically safe milk with raw-like properties and characteristics. Therefore, milk banks and researchers should be committed to develop novel pasteurisation technologies and procedures that can assure microbial inactivation, while preserving the nutritional, immunological and functional value of human milk.

High-pressure processing

Nowadays, increasingly demanding consumers require not only safe, but also high quality and fresh-like foods. This implies less extreme treatments and fewer or even no additives (Palou et al., 2007). There are several physical (e.g. heating, freezing, dehydration and packaging) and chemical (e.g. acidification and use of preservatives) food preservation methods, but the most commonly used is thermal treatment (e.g. pasteurisation and sterilisation). Even though heat treatment significantly reduces microbial levels and growth, it often results in undesired changes in foods, such as losses of colour, flavour, texture, smell and nutritional value, that is, an overall reduction of freshness and quality of the final product (Hogan et al., 2005). Therefore, new processing technologies are required to provide safe, fresher-tasting, nutritive foods with a reasonable shelf life and improved convenience (Ramirez et al., 2009). High-Pressure Processing (HPP) potentially answers many, if not all, of these challenges (Hogan et al., 2005).

HPP is a novel non-thermal processing technology of great interest in food research and industry, primarily as an alternative to thermal treatment. It consists in subjecting the desired product to pressures that frequently range from 100 to 1000 MPa, i.e., 1000 to 10 000 times atmospheric pressure (Huppertz et al., 2006).

The use of high pressure *per se* is not new; this technology has long been applied in various non-food industries, including production of plastics, ceramics, metal-forming and pharmaceutical tablet manufacture (Schaschke, 2011). Even today high pressure is still being studied for other innovative non-food applications (Oliveira et al., 2012; Salvador et al., 2010). HPP effects on foods were first studied in the late nineteenth century, when it was observed that processing milk at 670 MPa for 10 min achieved five to six microbial logarithmic reductions, extending shelf life up to 4 days after processing (Hite, 1899). HPP applied to foods has been studied for over 100 years, but the commercialisation of food products processed by HPP is fairly recent, as depicted in Table 6.

In the late 1980s, the desire for fresh long shelf life products in Japan brought to the market the first HPP product, a fruit jam (Table 6), as these needs could not be fully satisfied by any other existing technology (Patterson et al., 2006). Since then, a number of HPP foods from fruit juices to fish products like raw squid appeared in the Japanese markets (Hayashi, 1992). In the USA, foods such as avocados, oysters and other shellfish, and meat products have greatly benefited from high pressure in terms of fresher taste, extended shelf life and consumer safety. In Europe and in the USA HPP fruit juices are still one of the major commercial successes, but several other products and applications, like ready-to-eat (RTE) products with extended shelf life are also a major success now.

In just two decades HPP has clearly stood out from the set of food preservation emergent technologies, as reflected by the increasing number of high pressure units installed over the world (Figure 3). It is now a routine procedure for the processing of several commercialised foods. Current HPP foods on the market are mostly refrigerated or have reduced water activity and/or low pH to prevent bacterial spores germination, as HPP technology alone is limited to pasteurisation treatments (Mújica-Paz et al., 2011).

As shown in Figure 3, this technology is now increasingly used in industry for a wide range of products: vegetables, meats, seafood, fish, fruits, purees and others (e.g. dairy products). In addition, HPP is also being studied to promote modifications of functional properties of foods, like improved protein digestibility (Correia et al., 2011), while efforts are more and more made to develop new functional foods for demanding niche markets (Mújica-Paz et al., 2011). A prime example is the fat-free antibody rich colostrum beverage developed by Fonterra in New Zeland (COL+, 2009; Hembry, 2008). Because of its higher price, HPP products are still, yet less and less, targeted for certain niche markets with specific well-defined needs. A very recent example of this new approach is the case of the largest coffeehouse company in the world, Starbucks. This company acquired in 2011 Evolution Fresh in order to start a new business sector, Health and Wellness, by selling premium tasteful HPP pasteurised fruit juices (Food Ingredients First, 2011). Furthermore, Avure Technologies recently announced that the company will expand its manufacturing activity by building a new HPP equipments production plant within the year of 2012 (Table 6).

Regarding consumers acceptance of HPP technology, there are some recent studies reporting that, although the knowledge of what is the “high-pressure processing” is still scarce,

HPP has a very positive response and acceptance by the consumers when comparing with the other emerging processing technologies. Furthermore, when compared to thermally processed foods HPP products have a higher quality, which also contributes for its great consumer acceptance (Wright et al., 2007). Cardello et al. (2007) studied consumer perceptions of foods processed by diverse innovative and emerging technologies and concluded that irradiation and genetic modification resulted in the greatest negative response, meeting with consumer resistance, while high-pressure processing produced the most positive effect. On the other hand, Nielsen et al. (2009) studied consumer perception of HPP and pulsed electric field (PEF) for processing juice and baby food. These authors verified that consumers perceived the naturalness, improved taste and high nutritional value of HPP and PEF products, even though they lacked information about these technologies. When comparing the two, HPP showed a higher consumer acceptance.

Principles and operation

It is well-known that HPP (at refrigeration, ambient or moderate temperatures) inactivates vegetative pathogenic and spoilage microorganisms in foods with fewer changes in colour, flavour and texture than the conventional thermal methods (Cheftel, 1995). In this section the most important principles in which high pressure application effects are based, as well as the general HPP process will be described.

There are two fundamental scientific principles that are applied in the high-pressure processing of foods. The first is based on the Le Chatelier's Principle that states that when a system at equilibrium is disturbed, the system responds in order to minimise the disturbance

(Pauling, 1964). According to this, any (bio)chemical reaction, conformational change, or phase transition that is accompanied by a decrease in volume will be enhanced by pressure, while reactions involving an increase in volume will be inhibited (Cheftel, 1995). However, due to the complexity of foods and wide variety of phenomena that occur under pressure, it is difficult to predict HPP effects on foods (Palou et al., 2007).

The second is the principle of isostatic processing or the Isostatic Rule. This principle states that high pressure is uniformly and nearly instantaneously transmitted throughout the food (Torres and Velazquez, 2008), whether there is a direct contact with the pressure medium or the food is hermetically sealed in a flexible package that transmits pressure (Olsson, 1995). The food is compressed by uniform pressure from every direction (unlike heat processing where there are temperature gradients) and returns to its original shape when the pressure is released (see Figure 4). Thus, in contrast to thermal processing, the time necessary for pressure processing is independent of food geometry and size. Therefore, a number of advantages arise from this principle: treatment times can be and usually are short, the surface area of the food does not get over-processed, scaling of laboratory and pilot plant findings to commercial production are both simple and safe, and changes in the equipment or product packaging do not require new pressure and time conditions and process redesign (Mújica-Paz et al., 2011; Schaschke, 2011). None of these advantages is verified in thermal processes, as HPP transmission throughout a food is mass and time independent and heat transmission is not.

Moreover, when looking at the molecular level, pressure does not affect the covalent bonds of food components, and sensory properties, nutrients or bioactive compounds suffer no significant losses, again in contrast to the very often highly damaging effects of temperature.

Molecular compression is only capable of affecting the weaker bonds and forces, such as hydrogen bridges, electrostatic interactions and van der Walls forces, which explains the preservation effects of HPP (Mújica-Paz et al., 2011).

A high pressure system consists of a high pressure vessel and its closure, pressure-generation system, temperature-control device, and material-handling system (Mertens, 1995). Currently, most HPP machines used in the food industry are batch systems. Figure 5 presents three examples of modern HPP batch units. In a HPP batch system, after loading the vessel with the product(s) to be treated and closing it, the vessel is filled with the pressure-transmitting medium (e.g. water, as in industrial application on foods). Then air is removed from the vessel and high hydrostatic pressure is generated. High pressure is usually generated by direct or indirect compression, that is, either by reducing the volume of the pressure chamber inside the vessel using a piston for example or by pumping medium into the vessel (the most usual), respectively (Palou et al., 2007). When the desired pressure is reached the pump or piston is stopped and the pressure is maintained without further energy use. At the end of the hold time the system is depressurised, the vessel opened and the product unloaded (Hogan et al., 2005).

In batch systems the food product is generally treated in the final package to assure its maximum security (primarily against microbial contamination) until opening by the consumer. As the volume of foods decreases with compression and expands with decompression, HPP requires hermetic packages that can withstand a change of volume corresponding to the compressibility of the product (Hugas et al., 2002), without compromising seal integrity.

Vacuum-packed products are ideal for HPP. Generally, plastic packages are also suitable, while metal cans and glassware are not (Hogan et al., 2005).

High-pressure processing of human milk

HPP is the only emergent processing technology that has reached the markets of several countries with a variety of new products, because of its great advantages and consumers acceptance. However, regarding human milk there are only few reports on the effects of high pressure on its nutritional and microbial contents. Knowledge and fundamental understanding of pressure maintenance of macronutrients, immunological and functional agents, as well as inactivation of microorganisms and enzymes in human milk is still largely lacking. Thus, extensive investigation is required if one wants to make this a reliable alternative to the conventional Holder pasteurisation. To our knowledge, only four studies assessed HPP effects on human milk so far, those indicated in Table 7, where pressure application conditions and main results obtained are summarised.

The authors of these studies also performed Holder pasteurisation in order to compare the results obtained by both processing technologies. The experimental conditions and results of these studies will be presented in more detail in the following discussion topics. It is noteworthy that all the works so far published in literature regarding the effect of HPP on human milk are devoted to mature human milk from mothers who gave birth at term. There is not any published work investigating HPP effects neither on colostrum and transitional milk nor on preterm milk. Term milk in the early lactation stages (colostrum and transitional milk) and preterm milk are nutritionally richer than mature term milk, so also in this area more research is needed.

HPP effects on human milk components

Total protein, fat or carbohydrate contents after HPP have not been studied. There are also no studies concerning HPP effects on lactose, oligosaccharides or minerals and only one whey protein, one enzyme, two vitamins and thirty-eight fatty acids have been studied. As presented in Table 7, the human milk components that have been analysed before and after HPP are total IgA, lysozyme, vitamin C, tocopherols and fatty acids. Thereby, HPP effects on the overall nutritional composition and on other several antimicrobial and bioactive components should be investigated.

Whey proteins and indigenous enzymes

Viazis et al. (2007) were the first ones to study high-pressure processing of human milk. They investigated the effects of HPP on total IgA and lysozyme activity in comparison with Holder (or LTLT) pasteurisation. These two proteins have a remarkable antimicrobial activity and are significantly reduced after heating. Milk samples were processed at 400 MPa at 21 to 31 °C for 30, 60, 90 and 120 min (HPP) or at 62.5 °C for 30 minutes (Holder). HPP samples retained 85.6, 87.1, 80.6, and 75.4% of total IgA activity respectively, while LTLT pasteurised milk retained 51.2% activity. For lysozyme, pressurised human milk retained 106.9, 96.3, 96.3, and 95.8% activity respectively, while LTLT pasteurised milk retained 78.8% activity. These promising results indicated that HPP could be a better choice than Holder pasteurisation for pasteurising human milk. Even with treatments four times longer than Holder pasteurisation, HPP resulted in a higher maintenance of both proteins. After 120 min of HPP lysozyme activity decreased by 4.2%, but after 30 min of Holder pasteurisation its activity decreased 21.2%. For lysozyme even an increase in activity following treatment for 30 minutes (106.9% retention) was observed.

Later on, Permanyer et al. (2010) studied the effect of HPP treatments on human milk IgA retention at equal or higher pressure levels for a shorter time. These authors pressure processed human milk at 400, 500 and 600 MPa for 5 min at 12 ºC and determined IgA retention following the treatments, comparing it with the retention after Holder pasteurisation. It was observed that HPP samples treated at 400, 500 and 600 MPa retained 100, 87.9, and 69.3% of IgA respectively, and low-temperature long-time pasteurised ones retained 72%. These findings suggest that HPP at 400 MPa for 5 min at 12°C maintains the immunological protective activity associated with IgA antibodies, and even the pressure treatment at 500 MPa has higher IgA retention that the LTLT method.

Both of the works give valuable information about HPP capacity to better retain proteins with great significance for the infant's health (pressures ≤ 500 MPa). Currently, the only enzyme that has been analysed after HPP of human milk is lysozyme, but studies on the HPP of bovine milk reveal that most indigenous milk enzymes are quite baroresistant. As reviewed by Huppertz et al. (2006), most milk enzymes like plasmin, alkaline phosphatase, lactoperoxidase, xanthine oxidase, phosphoisomerase, gamma-glutamyltransferase and lipase are resistant to pressures up to 400 MPa.

High pressure effects on proteins (including enzymes) vary extensively with the protein being considered, the pressure, temperature and time of the processing, and the pH and composition of the food to be treated (Mújica-Paz et al., 2011). As pressure-treated proteins maintain their primary structure (because covalent bonds are not affected by HPP), the largest contribution to protein/enzyme inactivation comes from structural rearrangements of proteins under pressure. At pressures higher than 200 MPa changes in the tertiary structure of proteins

may occur and are due to changes of hydrophobic and ionic interactions (Balny and Masson, 1993).

Fatty acids

Molto-Puigmarti et al. (2011) recently performed the analysis of thirty-eight fatty acids (from C8:0 to C22:-n3) in human milk subjected to three HPP treatments and Holder pasteurisation. HPP treatments consisted in processing milk at 400, 500 and 600 MPa for 5 min at initial temperature of 21 °C. No statistically significant differences in the proportions of fatty acids were detected between pressurised, thermally pasteurised and untreated samples. Besides, pressure did not cause significant isomerisation of conjugated linoleic acid (CLA) in human milk. This indicates that infants receiving donor human milk treated by either of the two methods would not be deprived of fatty acids in general.

The authors could not guarantee that pressurisation was able to maintain the triglyceride structure intact, as HPP stability of human milk lipases has not been studied yet and the performed analysis quantified both fatty acids in lipid structures and free fatty acids (Molto-Puigmarti et al., 2011). There is evidence that LPL in bovine milk is quite resistant to high pressures (Pandey and Ramaswamy, 2004; Seyderhelm et al., 1996). In accordance with the described work, no changes in the proportions of fatty acids and CLA were found in high pressure homogenised cow's, ewe's and goat's milks (Rodríguez-Alcalá et al., 2009).

Vitamins

Molto-Puigmarti et al. (2011) studied HPP and Holder pasteurisation of vitamins C and E of human milk. The effects of pressure processing at 400, 500 and 600 MPa for 5 min (at 12 °C)

were compared to those of thermal pasteurisation at 62.5 °C for 30 min. Vitamin C (total vitamin C and ascorbic acid contents) and vitamin E (three tocopherol isomers: delta-, gamma- and alpha-tocopherols) were quantified (Molto-Puigmarti et al., 2011). No changes in vitamin C or ascorbic acid contents of milk were found after HPP, while Holder pasteurisation decreased them in 19.9 and 16.2%, respectively. Total vitamin C and ascorbic acid retentions were the same in the three HPP treatments. Regarding vitamin E, neither HPP nor Holder pasteurisation caused a significant decrease in the three tocopherols levels. These findings that HPP maintains vitamin C and tocopherol levels in human milk under the tested conditions agree with the idea that small molecules with no higher levels of structure, such as vitamins, are minimally or not affected by high pressure when samples are treated at mild temperatures (Balci and Wilbey, 1999).

HPP effects on bacteria in human milk

There are only two studies that assess HPP effects on vegetative bacteria present in human milk and both of them are very recent. The first investigated the efficacy of HPP for inactivation of five selected pathogens (Viazis et al., 2008), while the second evaluated HPP effects on total bacterial population in general and bacteria of the *Enterobacteriaceae* family in particular (Permanyer et al., 2010).

Viazis et al. (2008) chose the following pathogenic bacteria: *Listeria monocytogenes* ATCC 19115, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and ATCC 6538, and *Streptococcus agalactiae* ATCC 12927. These authors inoculated pooled donor human milk with each pathogen (10^8-10^9 CFU/mL) and subjected each one of them to a pressure treatment of 400 MPa at 21 to 31 °C for holding times that ranged from 0 to 50 min, depending

on the organism being treated. As in the previously mentioned studies, the results of this treatment were compared with those obtained after Holder pasteurisation. Holder pasteurisation resulted in a complete inactivation of all of the pathogens in human milk after 10 min. With respect to HPP, treatments of 2 and 4 min resulted in a complete inactivation of *L. monocytogenes* and *Strep. agalactiae* in human milk, respectively. However, the other pathogens were more difficult to inactivate. After 30 min of pressure treatment *Staphylococcus aureus* ATCC 25923 achieved an 8-$\log_{10}$ reduction and *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* were both reduced by 6-$\log_{10}$. Holder pasteurisation achieved higher levels of bacterial reduction for *Escherichia coli* and *Staphylococcus aureus* ATCC 25923 and ATCC 6538 when compared to HPP at 400 MPa. However, it should be noted that HPP for food pasteurisation is commonly used in industry at 500-550 MPa, pressures that cause higher microbial destruction. These facts point to HPP as a viable alternative for pasteurisation of human milk. The authors of this study also highlighted that it cannot be forgotten that HPP results in an overall improved nutritional quality of pasteurised human milk.

More recently, Permanyer et al. (2010) studied the bacterial load of human milk after HPP and Holder pasteurisation, recording total bacteria and *Enterobacteriaceae* counts. Raw milk samples presented a total bacterial count ranging from 1.3×10^2 to 2.9×10^4 CFU/mL and only one sample had a significant *Enterobacteriaceae* count of 3.0×10^1 CFU/mL. These samples were subjected to three HPP treatments of 400, 500 and 600 MPa for 5 min at 12 °C. The three HPP treatments reduced total bacterial and *Enterobacteriaceae* counts to undetectable levels, regardless of the initial bacterial load, as did Holder pasteurisation.

When comparing HPP treatment at 400 MPa in both works, although temperature is variable, it is possible to observe that the time necessary for vegetative bacteria inactivation is highly dependent of the initial contamination level (U.S. Food and Drug Administration, 2012), showing the importance of milk being collected and stored by the mothers in hygienic conditions and being handled in aseptic conditions in the HMB. Of the diverse bacterial communities in healthy human milk, the most abundant genera are *Streptococcus*, *Staphylococcus*, *Serratia*, *Pseudomonas* and *Corynebacteria* (Hunt et al., 2011), so more studies of HPP effects on these bacteria should be conducted, as they may (above certain levels) cause infections and diseases or, mainly in the case of *Pseudomonas* species, milk spoilage. However, several studies regarding HPP of bovine milk appear to indicate that Gram-positive species such as *L. monocytogenes* and *S. aureus* and Gram-negative *E. coli* are the most baroresistant of the investigated species (Huppertz et al., 2006) and HPP was able to effectively inactivate these pathogens in human milk (Viazis et al., 2008).

There is not any paper describing high pressure effects on bacterial spores, viruses, fungi or prions in human milk, since only vegetative bacteria have been analysed so far. Thus, further research is needed to evaluate the efficacy of HPP in the inactivation of these potential hazards for the babies health, mainly relevant viral pathogens and spore-forming bacteria.

Conclusion

Human milk has proven to provide the most appropriate nutritional, immunological and functional support for newborn infants. Besides the role that breastfeeding plays in the regular development of the infant, it also protects against infectious diseases in infancy and childhood.

The composition of human milk is not homogeneous over the course of lactation and colostrum is particularly rich in proteins with immunological functions. Furthermore, maternal diet is a key factor affecting human milk composition.

When the mothers cannot adequately supply their children, donated fresh milk is the recommended nutrition by the World Health Organization (WHO), being followed by pasteurised donated breast milk, preterm formula and ordinary formula as the last option. Therefore, human milk banks (HMB) are of an extreme importance for newborns and infants that cannot be breastfed. Nevertheless, currently applied Holder pasteurisation is primarily responsible for a number of losses of bioactive compounds and immune factors, significantly decreasing the benefits of mother's own milk.

According to the published studies on the high-pressure processing (HPP) effects in human milk, HPP has the potential to be developed into a successful pasteurisation method for human milk. It allows efficient inactivation of the studied microbial pathogens, while maintaining unique components that Holder pasteurisation diminishes and that are crucial to the healthy growth of neonates, infants and young children. There is, however, few available information describing HPP effects on the nutritional content of human milk. Fundamental knowledge is still lacking, so extensive research is required to make this a reliable alternative to the conventional thermal pasteurisation.

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Figure Captions

Figure 1. WHO hierarchy of infant feeding choices for low-birth-weight infants. Adapted from Arnold (2002).

Figure 2. Main practices of the process conducted in HMB.

Figure 3. Number of HPP equipments operating worldwide in the production of commercial food products. Courtesy of Hiperbaric.

Figure 4. The principle of isostatic processing. Courtesy of Avure Technologies Inc..

Figure 5. Examples of industrial HPP units of three of the companies manufacturing industrial-scale high pressure equipments. Courtesy of Avure Technologies Inc. (A), Uhde High Pressure Technologies (B) and Hiperbaric (C).

Tables

Table 1. Average basic nutrient composition of human milk in the three lactation stages (g/100 mL, unless otherwise indicated). Adapted from Emmett and Rogers (1997); Shi et al. (2011).

Nutrient	Colostrum		Transitional milk		Mature milk	
	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)
Water	88.2	-	87.4	-	87.1	-
Total solids	-	11.58	-	11.37	-	11.49
Total nitrogen	0.31	1.38	0.23	1.33	0.20	1.27
Non-protein nitrogen	-	0.05	-	0.05	-	0.07
Protein	2.0	1.33	1.5	1.28	1.3	1.20
Lipid	2.6	3.45	3.7	3.29	4.1	3.04
Carbohydrate	6.6	-	6.9	-	7.2	-
Lactose	-	6.79	-	6.65	-	6.97
Energy [a]	56	-	67	-	69	-
Ash	-	0.17	-	0.20	-	0.21

[a] (kcal/100 mL); "-" means data not reported.

Table 2. Main whey protein composition of human milk in the three lactation stages (mg/mL) (Shi et al., 2011).

Protein	Colostrum	Transitional milk	Mature milk
α-lactalbumin	1.38	1.33	1.27
Lactoferrin	3.04	3.01	2.05
Serum albumin	0.27	0.30	0.19
IgA	1.48	1.07	0.33
IgG	0.46	0.22	0.19
IgM	0.12	0.09	0.08

Table 3. Average fatty acid composition of human milk in the three lactation stages (%, unless otherwise indicated).

Fatty acid	Colostrum		Transitional milk		Mature milk	
	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)
C10:0	-	1.17	-	1.04	-	0.98
C12:0	-	4.55	-	4.43	-	4.15
C14:0	-	4.82	-	4.81	-	4.60
C16:0	-	21.95	-	22.94	-	24.38
C18:0	-	5.63	-	6.03	-	5.97
C20:0	-	0.12	-	0.13	-	0.13
C22:0	-	0.12	-	0.10	-	0.14
Total saturated	1.1[a]	38.36	1.5[a]	39.48	1.8[a]	40.35
C14:1	-	Tr.	-	Tr.	-	0.24
C16:1	-	3.05	-	3.29	-	3.33
C18:1	-	30.27	-	33.16	-	30.92
C20:1	-	0.60	-	0.76	-	0.57
C22:1	-	0.70	-	Tr.	-	0.22
Total MUFA	1.1[a]	34.62	1.5[a]	37.21	1.6[a]	34.65
C18:2	-	18.88	-	16.51	-	16.97
C18:3 γ	-	0.37	-	0.21	-	1.08
C18:3 α	-	5.27	-	3.39	-	3.29
C20:2	-	0.35	-	0.36	-	0.37
Total PUFA	0.3[a]	24.87	0.5[a]	20.47	0.5[a]	21.71
Cholesterol	31[b]	-	24[b]	-	16[b]	-

[a] (g/100 mL); [b] (mg/100 mL); "-" means data not reported; Tr. means trace amounts.

Table 4. Average vitamin composition of human milk in the three lactation stages (mg/100 mL, unless otherwise indicated).

Vitamin	Colostrum		Transitional milk		Mature milk	
	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)
Vitamin C	7	1.7[a]	6	2.2[a]	4	1.6[a]
Folate	2[b]	4.7[c]	3[b]	4.6[c]	5[b]	2.4[c]
Pantothenate	0.12	204.5[c]	0.20	186.0[c]	0.25	249.3[c]
Biotin [b]	Tr.	-	0.2	-	0.7	-
Niacin	0.1	180.0[c]	0.1	173.8[c]	0.2	182.7[c]
Riboflavin	0.03	16.9[c]	0.03	17.6[c]	0.03	13.7[c]
Thiamin	Tr.	6.5[c]	0.01	5.2[c]	0.02	6.3[c]
Vitamin B_6	Tr.	5.4[c]	Tr.	4.5[c]	0.01	4.6[c]
Vitamin B_{12} [b]	0.1	-	Tr.	-	Tr.	-
Retinol	155[b]	24.1[d]	85[b]	31.9[d]	58[b]	20.4[d]
Vitamin D	-	159.7[e]	-	97.6[e]	0.04[b]	0.2[e]
Vitamin E	1.30	294.4[c]	0.48	174.5[c]	0.34	234.6[c]
Vitamin K [c]	-	22.4	-	22.7	-	0.8

[a] (mg/100 g); [b] (µg/100 mL); [c] (µg/100 g); [d] (IU/100 g); [e] (IU/100 g^{-1}); "-" means data not reported; Tr. means trace amounts.

Table 5. Average mineral composition of human milk in the three lactation stages (mg/100 mL, unless otherwise indicated).

Mineral	Colostrum		Transitional milk		Mature milk	
	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)	Emmett and Rogers (1997)	Shi et al. (2011)
Ca	28	28.5	25	28.0	34	33.4
P	14	14.1	16	15	15	16.7
Cl	-	84.1	86	89.7	42	88.8
Mg	3	3.5	3	3.6	3	3.7
K	70	46.0	57	46.3	58	50.4
Na	47	11.0	30	16.3	15	13.8
Cu	0.05	61.3[a]	0.04	59.0[a]	0.04	36.7[a]
Fe	0.07	0.05	0.07	0.05	0.07	0.05
Zn	0.6	0.3	0.3	0.3	0.3	0.2
I [a]	-	22.1	-	33.1	7	27.9
Se [a]	-	1.4	2	1.9	1	1.5

[a] (μg/100 mL); "-" means data not reported.

Table 6. Landmark events in the history of HPP for food products. Adapted from Patterson et al. (2006) and supplemented with informations from Food Ingredients First (2011); Nguyen et al. (2010); Tonello (2011).

Year	Event(s)
1895	Royer (France) used high-pressure to kill bacteria experimentally
1899	Hite (USA) used high-pressure for food preservation
1980s	Japan started producing high-pressure jams and fruit products
1990s	Fresherized Foods (formerly Avomex; USA) began to produce high-pressure guacamole from avocados with a fresh taste and extended shelf life
2000	Mainland Europe began producing and marketing fresh fruit juices (mainly citrus) and delicateness-style cooked meats. High-pressure self-sucking oysters, poultry products, fruit juices and other products were marketed in the USA
2001	HPP fruit pieces given approval for sale in the UK. Launch of the first HPP fruit juices in the UK
2003	Espuña (Spain) launched a line of ready-to-microwave HPP meat snacks (e.g. bacon and cheese rolls). In 2005, the company developed the first high-pressure sliced cured ham.
2008	Fonterra (New Zeland) developed a pressurised antibody rich colostrum beverage
2009	FDA approval of the pressure-assisted thermal processing (PATP) for production of shelf-stable low acid foods
2011	Starbucks aquired Evolution Fresh with the aim of bringing premium HPP juices to the marketplace
2012	Avure Technologies announced the expansion of its manufacturing capacity by building a new facility in the USA at the end of 2012.

Table 7. Studies of HPP effects on diverse mature human milk components, including bacterial content.

Reference	Components	HP Treatment	Conclusions*
Viazis et al. (2007)	Immunoglobulin A, lysozyme activity	400 MPa for 30 to 120 min at 21 to 31 °C	HPP samples retained significantly higher levels of IgA and lysozyme activity than Holder's
Viazis et al. (2008)	Inactivation of five selected bacterial pathogens	400 MPa for 0 to 50 min at 21 to 31 °C	Effective in reducing the bacterial population; *E. coli* and *S. aureus* ATCC 6538 are the most pressure-resistant (6-$\log_{10}$ reduction after 30 min)
Permanyer et al. (2010)	Immunoglobulin A, bacterial load	400, 500 or 600 MPa for 5 min at 12 °C	400, 500 and 600 MPa: IgA retention of 100, 87.9 and 69.3% (Holder: 72%); reduction of bacteria to undetectable levels
Molto-Puigmarti et al. (2011)	Vitamin C, fatty acids and tocopherols	400, 500 or 600 MPa for 5 min at 12 °C	Fatty acids and tocopherol did not vary with HPP and Holder, vitamin C was maintained after HPP (Holder: - 20%)

*Holder stands for Holder pasteurisation (62.5 ºC for 30 minutes).

Figures

Figure 1

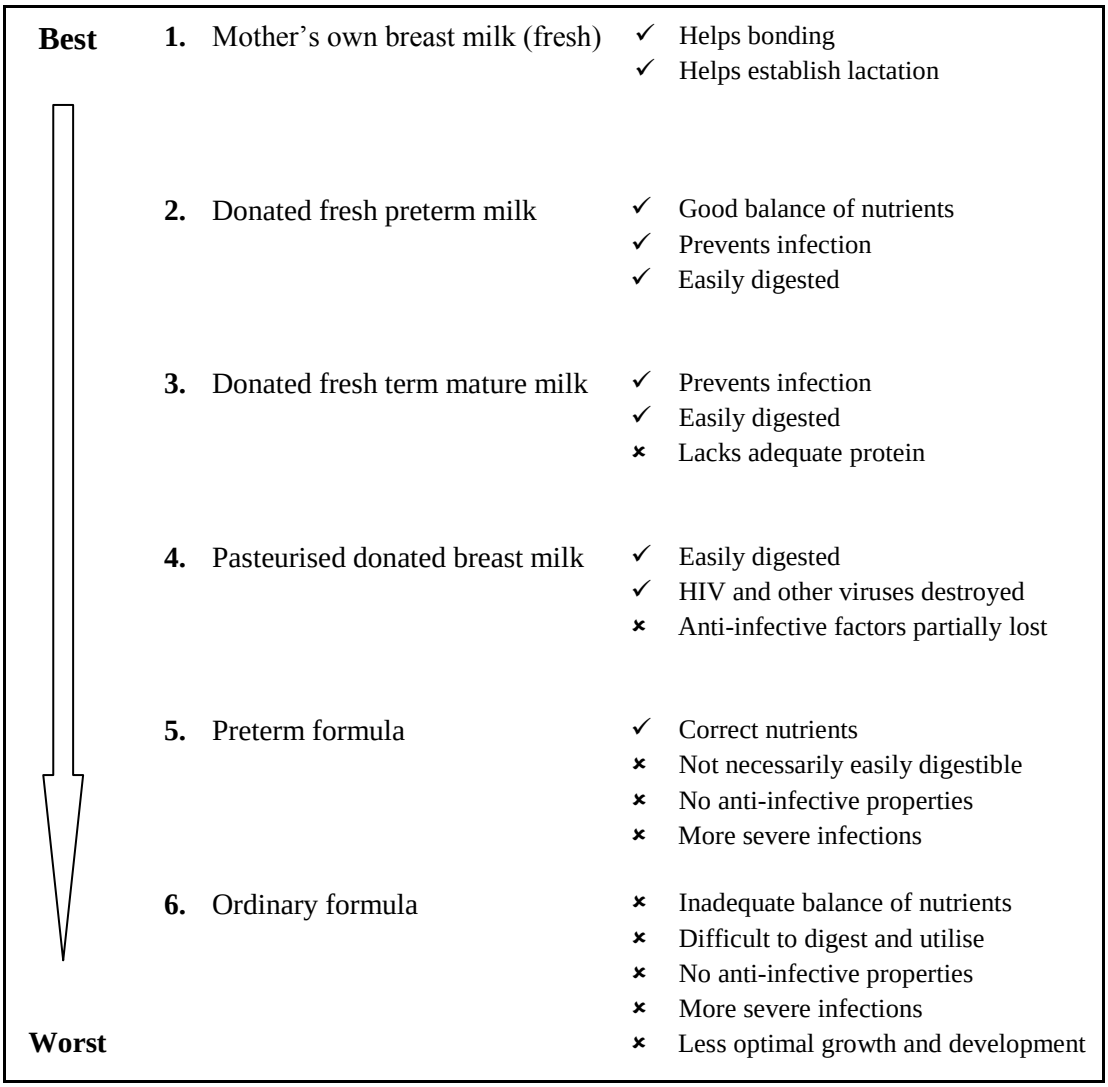

Figure 2

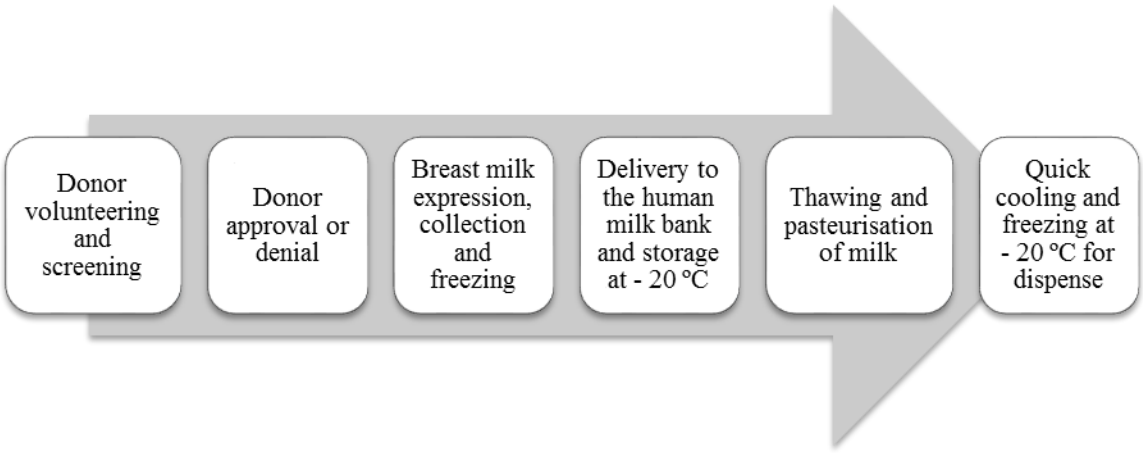

Figure 3

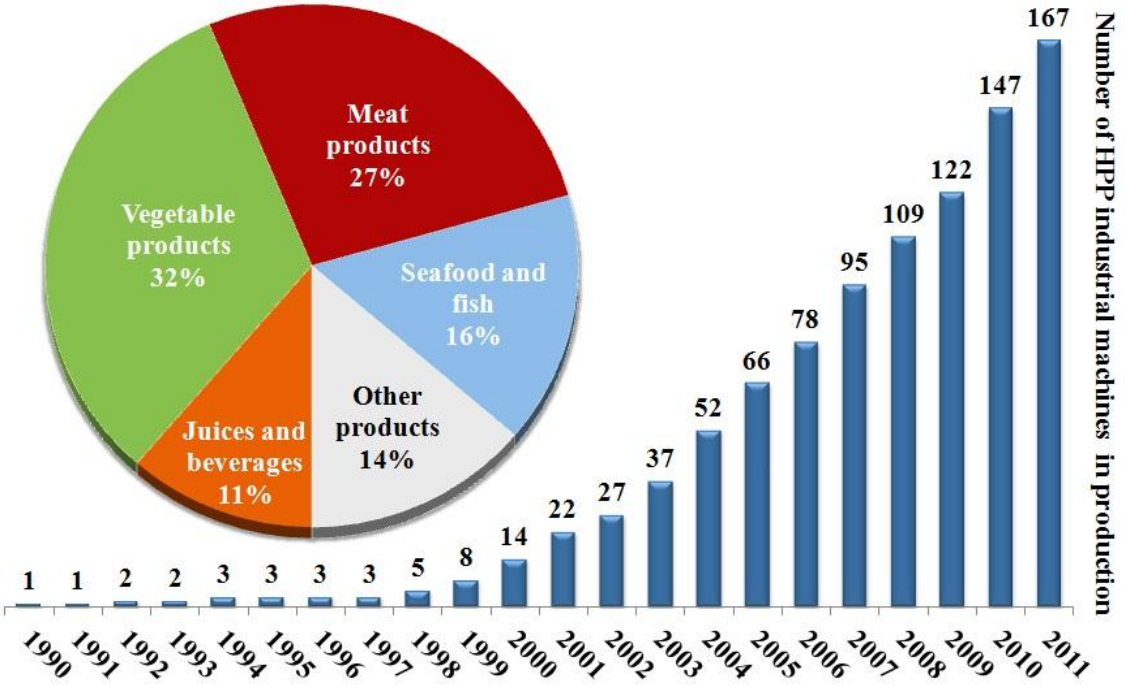

Figure 4

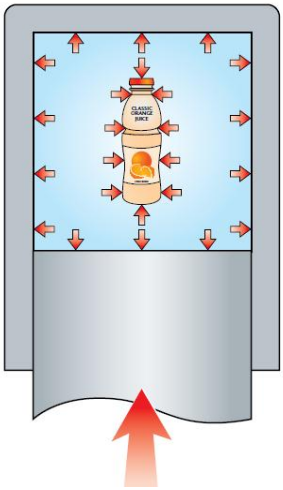

Figure 5