Volatile profile of breast milk subjected to high-pressure processing or thermal treatment

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Abstract

The effect of Holder pasteurisation (HoP) (62.5 °C for 30 min) or high-pressure treatments (400 or 600 MPa for 3 or 6 min) on the volatile compound profile of human breast milk was evaluated, in order to compare both preservation technologies. A total of 46 different volatile compounds was found in milk samples. The most abundant compounds detected were aliphatic hydrocarbons. In general, the effect of some high-pressure treatments on the volatile profile of human milk was less intense than that caused by HoP. The treatments at 400 and 600 MPa for 3 min maintained the volatile compounds at similar levels to those found in control milk samples. However, the application of 600 MPa for 6 min changed the original volatile compounds of human milk, even more than HoP. Since, HPP at 400 or 600 MPa for 3 min preserved the original volatile compounds of human milk, this novel process may be an alternative to thermal pasteurisation.

Keywords: holder pasteurization, high-pressure technology, human milk, volatile compound.

1. Introduction

It is worldwide assumed that breastfeeding exerts positive effects on the general health, growth and development of infants. Apart from nutrient components, human milk possesses bioactive and immune factors that provide adequate host defence against infections, actively modulate immune responses, and favourably modify intestinal bacterial colonisation (Diehl-Jones & Askin, 2004; Rosetta & Baldi, 2008). When breastfeeding is not possible, donor milk from human milk banks is considered the best alternative to the mother's own milk, since breast milk is better tolerated than infant formulas, especially, for preterm infants (Leaf & Winterson, 2009).

To eliminate potential pathogenic microorganisms and hence assure microbiological safety, banked human milk is pasteurised prior to distribution, using low-temperature (65 °C) long-time (30 min) thermal pasteurization, also known as Holder pasteurisation (HoP) (Hartmann, Pang, Keil, Hartmann, & Simmer, 2007). However, this method partially interferes with the immunological and nutritional properties of breast milk, thus reducing some important compounds, such as immunoglobulin A (IgA), IgM, IgG, trace elements and some vitamins, among other biological components (Ewaschuk, Unger, Harvey, O'Connor, & Field, 2011; Moltó-Puigmartí, Permanyer, Castellote, & López-Sabater, 2011). Likewise, HoP diminishes the antioxidant activity of milk (Romeu-Nadal, Castellote, Gayà, & López-Sabater, 2008). High-pressure processing (HPP) may be a promising alternative for pasteurisation of banked human milk. This non-thermal process is an emerging food processing method that can be applied to solid and liquid foods to provide microbiologically safe, nutritionally intact, and organoleptically high-quality products (Viazis, Farkas, & Jaykus, 2008). This technology inactivates pathogenic

microorganisms by applying hydrostatic high pressure (usually 400–800 MPa) to food for short-term treatments (less than 5–10 min). In general, chemical composition and sensory analysis of food processed by high pressure are similar to untreated products in colour and aroma, and changes are less intense than that caused by heating treatment (Kouniaki, Kajda, & Zabetakis, 2004).

Lysozyme is a bioactive compound with antimicrobial activities that contributes to maintaining the microbiological quality of milk. Interestingly, it has been reported that HPP (400 MPa) preserves this compound better than HoP processing (Viazis, Farkas, & Allen, 2007). It has also been demonstrated that certain HPP treatments are more suitable than pasteurisation to preserve vitamin C and E content in human milk (Moltó-Puigmartí, Permanyer, Castellote, & López-Sabater, 2011). Similar effects have been found for cytokine concentration of human milk, which are better maintained by HPP than HoP (Delgado, Cava, Delgado-Adámez, & Ramírez, 2014).

It is generally accepted that the volatile profile (particularly odorous volatiles) reflects the image of the odour and aroma of milk. Human milk aroma has been shown to be composed of a delicate balance of diverse odorants from different substance classes, such as fatty acids, terpenoid substances, saturated and unsaturated aldehydes and ketones, among others (Buettner, 2007; Spitzer & Buettner, 2009). Numerous factors influence the aroma of milk, ranging from food or odorant formulations ingested by the mother (Hausner, Bredie, Mølgaard, Petersen, & Møller, 2008) to storage conditions (Spitzer & Buettner, 2009). In fact, formation of intense off odours, such as fishy, metallic and hay-like smells is characteristic of particular storage conditions (Spitzer & Buettner, 2010; Spitzer, Doucet, & Buettner, 2010). Another typical aroma, which reflects low quality

breast milk, is rancid odour, which is a result of short to medium-chain fatty acids (C4–C12) produced predominantly by the action of bacterial lipases on milk triglycerides (Lawrence, 1999). The aforementioned sensory alterations should be carefully considered in relation to the specific olfactory skills of neonates, since they are able to recognise specific smells with high reliability and to be greatly receptive for flavour learning (Mennella, Jagnow, & Beauchamp, 2001). For these reasons, the preservation of the original volatile flavour compounds in human milk is an important concern, since it influences the quality of the breast milk and, consequently, the infant feeding. Moreover, the analysis of volatile flavour compounds of breast milk provides information about global changes that occur after processing and if adverse reactions appear (e.g., lipid oxidation, etc.). Volatile compound analysis has been proposed as a suitable technique for comprehensive assessment of the effect of novel technologies and to compare them with thermal treatment (Vervoort et al., 2012). This methodology also allows us to evaluate both treatments generally, to assess the potential benefits of each.

Taking everything into account, the purpose of this study was to evaluate the effect of HPP and HoP techniques on the volatile profile of human milk, in order to elucidate which is the most appropriate technology to preserve the original volatile profile of milk stored in milk banks.

2. Material and Methods

2.1. Human milk collection

Mature human milk samples were collected from healthy volunteers ($n = 8$; between 32 and 36 years-old and 3–26 months from parturition) from Mère Association (Association

of Breastfeeding Support of Badajoz, Spain) in July 2012. Each mother donated 50–150 mL of milk that they extracted during the day before the application of the different treatments. These samples were kept in sterile containers and refrigerated (domestic refrigerator) until they were transported (on the afternoon of the same day) to Technological Agri-Food Institute (CICYTEX-Intaex), where they were also maintained under refrigeration. The experiments were carried out the next morning. Through this period, an unbroken cold chain was guaranteed.

Before processing, all milk donations were mixed under sterile conditions (under a laminar flow cabinet; Telstar AV-100[®]) reaching a final volume of 700–800 mL. Milk was mixed for a better evaluation of the effect of processing (HoP and HPP treatments). Milk aliquots (10 mL) were vacuum-packed in polyethylene bags (9.3 mL O₂/m²/24 h at 0 °C) for HPP or divided into glass tubes for HoP treatment. Control samples were also vacuum-packaged in plastic bags. Finally, all processed or unprocessed (control) samples were stored at –80 °C (all at once) until their analysis, which was carried out within one month.

Milk samples were protected from light by using aluminium foil during sample manipulation. The time between mixing milk, packaging, processing and freezing was less than 3 h.

2.2. Thermal treatment

Thermal treatment (HoP) was applied in sterile glass tubes containing 10 mL of milk which were heat-treated at 62.5 °C for 30 min in a water bath (Agibat-20, Selecta, Barcelona, Spain) under constant agitation. Five screw tubes ($n = 5$) with milk samples were processed and another five tubes ($n = 5$) with breast milk that we had stored were

used as temperature control references of the temperature of the process (those latter tubes were not included in the study). When the temperature registered in the bottle reached 62.5 °C, the process continued for 30 min. After the treatment, the tubes were submerged in cold water to quickly reduce the temperature.

2.3. High pressure processing

The milk was pressurised in a semi-industrial discontinuous hydrostatic press machine of 55 L capacity (Hiperbaric Wave 6000/55; Burgos, Spain) with a maximum working pressure of 600 MPa. Samples were treated at 400 or 600 MPa for 3 or 6 min ($n = 20$; five samples for each treatment condition). These samples were compared with control ($n = 5$) and with thermally treated milk ($n = 5$). Initial water temperature, used as the pressure-transmitting fluid, in the vessel was 10 °C. Times to reach 400 MPa and 600 MPa were 174 s and 230 s, respectively. Industrial equipment does not allow the control of temperature changes during the pressurisation process. According to the human milk composition (water 88%, fat 3.5%, protein 1% and carbohydrate 7.5%), the theoretical increase of temperature due to adiabatic heating was 3 °C/100 MPa (Patazca, Koutchma, & Balasubramaniam, 2007).

2.4. Characterisation of the volatile profile in human milk

The profile of volatile compounds was determined by SPME followed by GC-MS. This technique has been used widely to extract many volatile and semi-volatile organic components in dairy products (Chin, Bernhard, & Rosenberg, 1996; Jaillais, Bertrand, & Auger, 1999; Shooter, Jayatissa, & Renner, 1999; Marsili, 1999; Contarini & Povolo, 2002; Frank, Owen, & Patterson, 2004), and possesses several advantages compared to other traditional solid-phase extractions (Marsili, 1999; Vas & Vekey, 2004;).

Eight grams of human milk were placed in a 50-mL vial. One analysis was performed for each sample. The sample was stirred for 30 min at 35 °C to accelerate equilibration of headspace volatile compounds between the milk matrix and the headspace. Then, volatile compounds were extracted by placing a 1-cm 50/30 µm divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, Bellefonte, PA) into the vial and exposing it to the headspace for 30 min at 35 °C. After extraction, samples were directly desorbed into the injection port of the GC which was at 270 °C. The analyses of volatile compounds were performed on a Varian CP-3800 GC gas chromatograph coupled to a Varian Saturn 2200 MS (Varian Inc., Palo Alto, CA). Volatile compounds were separated using a capillary column (HP-5; 50 m × 0.32 mm ID, 1.05 µm film thickness; Agilent, Santa Clara, CA). The carrier gas was helium with a flow of 1 mL/min. Samples were injected into the liner in splitless mode. The temperature programme was isothermal at 40 °C for 10 min, and then raised at 5 °C/min to 240 °C and held for 11 min. The GC–MS transfer line temperature was 280 °C. The MS operated in electron impact mode with electron impact energy of 70 eV; and collected data at a rate of 0.7 scans/s over a range of m/z 40–650. The majority of compounds were identified by comparison with mass spectrum and retention time of commercial reference compounds provided by Sigma–Aldrich (St. Louis, MO). These compounds were 2,3,4-trimethylpentane, 2,2,4-trimethylhexane, octane, decane, dodecane, tetradecane, α -pinene, D-limonene, toluene, *p*-xylene, hexylbenzene, octanoic acid, decanoic acid, dodecanoic acid, hexanal, heptanal, octanal, 2-furancarboxaldehyde, 5-(hydroxymethyl)-2-furancarboxaldehyde, 3-heptanone, 2-heptanone, 2(5*H*)-furanone (γ -crotonolactone), and 3-hydroxy-2-methyl-4*H*-pyran-4-one (maltol). The remaining

compounds were identified by comparison of their mass spectra with those contained in the NIST and Varian mass spectral libraries. The integration of peak areas was performed using Varian Workstation[®] software (version 6).

2.5. Statistical analysis

The differences in the volatile profile of breast milk after processing were analysed using one-way analysis of variance (ANOVA). HSD Tukey's test was applied to compare the mean values of the volatile compounds. Mean values ($n = 5$) and standard error of the mean (SEM) are reported. All these statistical treatments were performed using the SPSS program for Windows, version 17.0 (SPSS Inc., Chicago, IL).

3. Results and Discussion

The preservation of original volatile compounds in human milk is an important concern, since variations in the profile of these compounds may negatively affect its quality. In fact, global acceptance of human milk by neonates is directly correlated with its aroma, a parameter influenced by odorous volatile compounds present in this biological matrix. In this respect, several studies have shown that newborns are very sensitive to olfactory changes, being able to recognize specific odours with high reliability (Terry, 2004; Martínez-Costa, Silvestre, López, Plaza, Miranda, & Guijarro, 2007).

Table 1 shows the volatile compounds (represented by arbitrary area units AAU $\times 10^3$), grouped by chemical families, detected in the headspace of human milk samples subjected to different preservation treatments (HoP and HPP). Figure 1 shows total AAU per chemical group. A total of 46 different volatile compounds was found in milk samples. The most abundant compounds detected were aliphatic hydrocarbons (12

alkanes and cycloalkanes and 2 terpenes). There were also 4 aromatic hydrocarbons, 5 carboxylic acids, 8 aldehydes, 4 ketones, 6 furans and pyrans and 5 alcohols.

The results show that the levels of the vast majority of these compounds (i.e., 40 out of 46) exhibited significant statistical differences ($p < 0.05$) after the application of preservation treatments, with respect to their corresponding initial values, indicating that the application of the different treatments affected the volatile profile of human milk. It is important to note that these variations were dependent on the type of treatment and the pressure intensity applied.

3.1. Hydrocarbons

A total of 18 hydrocarbons were detected in the human milk studied. They were grouped into aliphatic hydrocarbons (8 alkanes, 4 cycloalkanes and 2 terpenes) and aromatic hydrocarbons (4 compounds).

3.1.1. Aliphatic hydrocarbons

Alkanes and cycloalkanes were the most abundant compounds detected in the headspace of milk, both in control and treated samples at 400 or 600 MPa for 3 min. The levels of these volatile compounds remained constant after the application of HPP at short times (3 min); however, at longer times (6 min), the levels of these compounds were reduced. These reductions were even more noticeable after the HoP treatment. Curiously, neither HoP nor HPP treatments affected the concentration of the aliphatic hydrocarbons octane and dodecane in human milk.

Numerous studies have reported the presence of aliphatic hydrocarbons in processed milk. Pereda et al. (2008) detected the presence of 19 alkanes in cow milk. In that study, the effect of ultra-high-pressure homogenisation on the volatile profile of cow milk was

compared with thermal treatments. Likewise, several studies have detected aliphatic hydrocarbons in ultra-pasteurised cow milk (Solano-López, Ji, & Álvarez, 2006), in pasteurised and fermented cow milk (Imhof & Bosset, 1994) and also in fresh cow milk (Toso, Procida, & Stefanon, 2002). Given that both the origin and the effects derived from the application of different conservation techniques on these compounds remain unclear, some authors have suggested that these effects could be influenced by mothers' diet (Pereda et al. 2008).

Storage conditions also influence the volatile compounds profile of milk. In fact, it has been proven that sensory changes in the aroma profile of human milk occur during storage at different freezing temperatures (Spitzer & Buettner, 2010; Sandgruber, Much, Amann-Gassner, Hauner, & Buettner, 2012). Despite the fact that these reports detected low levels of alkanes and cycloalkanes, our results show the presence of high levels of different alkanes and cycloalkanes in milk. These differences could be due to different extraction methods of volatile compounds in the studies (SPME *vs.* solvent-assisted flavor evaporation).

A cyclic hydrocarbon, 1,2,4-trimethylcyclopentene, was the most abundant compound identified in the control milk samples. However, after the thermal treatment (HoP) neither this compound nor any other cycloalkane was detected. So far, no article has given explanation for this issue, although a justification for this reduction may be that these compounds had been transformed into other complex volatile compounds during the process.

Interestingly, the terpenes detected showed the highest concentration after HoP treatment. Based on our results, the formation and/or liberation of these compounds could be

enhanced by heat treatment. In particular, the initial levels of D-limonene were increased (>70 %) after HoP whereas HPP treatments did not modify the initial levels. The origin of D-limonene is related to the mother's diet; in particular, this compound would be a component of vegetables consumed (Sun, 2007). However, other authors have reported modifications of its concentration due to processing. Pereda et al. (2008) found differences in this compound between raw, high-pasteurised (90 °C, 15 s) and ultra-high-pressure homogenisation (UHPH; 200–300 MPa, 30–40 °C)-treated cow milk, limonene being most abundant in UHPH-treated milk. Limonene has also been described by Contarini et al. (1997) in pasteurised (78 °C, 15 s), ultra-high-temperature processed (UHT; 144–148 °C, 3.5 s) and in-bottle sterilised milk (120 °C, 30 min), its concentration being most abundant after UHT treatment. None of these authors provided an explanation for the variations observed in this compound in human milk. Based on these previous works, we cannot confirm that the formation/liberation of this compound in our study was enhanced by heat treatment.

α -Pinene, a chemical component that belongs to bicyclic monoterpenoids, was identified in human milk. This compound only appeared in the milk treated by HoP. Mounchili et al. (2005) have associated this terpene with earthy, burnt, bread/caramel-like, sulfur-like, sour-like, and rancid aromas. The same as D-limonene, the origin of α -pinene could be from vegetables consumed by mothers.

3.1.2. Aromatic hydrocarbons

In general, aromatic hydrocarbons were reduced by over 70 % after the application of thermal treatment. By contrast, they remained almost constant with respect to the initial values when HPP treatments were applied.

Toluene concentration was maintained throughout all the treatments applied (HoP and HPP). This compound has been identified in cow milk by numerous authors (Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001; Mounchili et al., 2005; Pereda et al., 2008; Contarini et al., 1997). It may originate from the degradation of carotenoids present in fresh milk (Mounchili et al., 2005; Contarini et al., 1997). However, Kim, Halden, and Juckle (2007) consider toluene present in human milk as an environmental pollutant. Mounchili et al. (2005) concluded that sulfur, rancid, burnt and sweaty odour attributes of human milk are provided by toluene.

3.2. Carboxylic acids

The carboxylic acid content suffered a relevant increase after applying HPP at longer times (400 MPa or 600 MPa for 6 min). This enhancement is associated with the release of short-chain fatty acids resulting from the degradation of human milk triglycerides (Pereda et al., 2008). Substances involved in fat oxidation processes are linked with the appearance of potent odorants (e.g., rancid, sweaty or soapy aroma) (Spitzer & Buettner, 2010; Pereda et al., 2008). Similarly, Mounchili et al. (2005) connected burnt milk odour with the presence of octanoic acid and decanoic acid. Consequently, an excess of these compounds would probably produce negative attributes in human milk.

Although previous studies (Spitzer & Buettner, 2009; 2010; Spitzer, et al., 2010), have detected numerous carboxylic acids in human milk, only 5 carboxylic acids were found in the current study. This may be due to the fact that these components are less volatile than others, such as aldehydes or ketones. The temperature utilised in our experiments for extracting the volatile compounds (35 °C) could inhibit the extraction of carboxylic acids. In fact, González-Córdova and Vallejo-Córdova (2001) concluded that the most

convenient conditions for extracting free fatty acids in human samples by SPME procedure were 70 °C and the use of a polyacrylate fibre.

3.3. Aldehydes

A total of 8 aldehydes were identified in human milk. The chromatographic signal matching octanal was not resolved correctly due to its overlapping with another compound, 2*H*-pyran-2,6(3*H*)-dione. For this reason, we decided to quantify both compounds together as octanal + 2*H*-pyran-2,6(3*H*)-dione.

Levels of aldehydes in milk increased with processing. This increase was 40–50% in high-pressure treated samples at short times (3 min), such an increase being higher in milk samples treated with HoP and HPP at longer times (6 min). The largest enhancement was observed in the samples treated by HPP at 600 MPa for 6 min, where the total area of aldehydes showed an 18-fold increase over control. Among these aldehydes, hexanal, octanal + 2*H*-pyran-2,6(3*H*)-dione, 2,5-furandicarboxaldehyde, 5-(hydroxymethyl)-2-furancarboxaldehyde and (*E*)-2-decenal, were compounds that mainly contributed to the aforementioned increase. No statistical differences were found in other aldehydes levels, such as 2-furaldehyde, heptanal or (*E*)-2-heptenal, with respect to their initial values. Nevertheless, 2-furaldehyde concentration was clearly enhanced after HPP treatment at 600 MPa for 6 min.

Hexanal, heptanal, (*E*)-2-heptenal, octanal and (*E*)-2-decenal are produced in lipid oxidation processes (Nursten, 1997; Elisia & Kitts, 2011). However, 2-furaldehyde, 2*H*-pyran-2,6(3*H*)-dione, 2,5-furandicarboxaldehyde and 5-(hydroxymethyl)-2-furancarboxaldehyde originate from sugars and amino acids *via* Maillard reaction (Namiki, 1988; Friedman, 1996). The volatile compounds derived from lipid oxidation

generally originate from the oxidation of fatty acids present in milk, particularly, the unsaturated fatty acids, which are more susceptible to degradation (Vázquez-Landaverde, Torres, & Qian, 2006). Based on our results, the increase of compounds derived from lipid oxidation, such as aldehydes and ketones, could be correlated with the diminution of hydrocarbons observed in milk treated with HPP at longer times and thermal procedures. On the other hand, the formation of volatile compounds arising from Maillard reaction is dependent on the abundance of lactose, which represents the main (90%) carbohydrate in human milk (Northrop-Cleves, 2001).

The aldehyde hexanal is a product generated by the oxidation of linoleic acid (Belitz et al. 2009). Elisia and Kitts (2011) observed that the quantification of levels of hexanal in human milk can be used as a reliable indicator of the oxidative status of the milk. In our study, the levels of this compound were significantly higher in milk treated at 600 MPa for 6 min compared to samples treated at 400 MPa for 6 min and to pasteurised milk samples. Nevertheless, hexanal concentration in control groups as well as groups pressurised for 3 min showed intermediate values. These results are in accordance with those obtained by Elisia and Kitts (2011), since they concluded that the concentration of hexanal did not change after HoP processing. Furthermore, they found a link between the content of tocopherol and ascorbic acid in milk with its oxidative stability (represented by its hexanal content).

In a previous study, we evaluated the effect of HPP (400 or 600 MPa for 3 or 6 min) on three tocopherol isomers (alpha, gamma and delta) present in human milk (Delgado et al., 2013). Human milk pressurisation at 600 MPa and HoP significantly decreased the concentrations of tocopherols compared with the control. The decrease of tocopherol in

samples treated at 600 MPa could be related to the development of lipid oxidation reactions because hexanal significantly increased in human milk pressurised at 600 MPa for 6 min. However, hexanal levels found in HoP milk were similar to those in control milk; a higher hexanal level (oxidation) in HoP milk might be expected, which could be associated with the decline of tocopherol reported in these samples. The detection and quantification of other antioxidants, such as ascorbic acid or the evaluation of fatty acids profile and the physical distribution of lipids in fat globule in processed milk could explain these results.

Our research group has also evaluated changes in the fatty acid profile of human milk after HoP treatment and HPP processing (Delgado et al., 2014). We observed a noticeable decrease in the main polyunsaturated fatty acids (the most prone to oxidation) of human milk, in particular, those samples treated at 600 MPa for 6 min. It was hypothesised that the elevated content of oxidised compounds (aldehydes) in HPP treated milk (especially, at 600 MPa for 6 min) could be due to the fracture of fat globules where lipids were immersed, as a result of pressure intensity. Studies have demonstrated that creaming of refrigerated cow's milk is lower in pressurised milk (at 400 and 600 MPa) than in non-processed milk (Huppertz, Fox, & Kelly, 2003). Consequently, HPP treatment modifies some properties of fat present in human milk, which may be due to changes in fat globules and/or other milk constituents.

The compound 5-(hydroxymethyl)-2-furancarboxaldehyde originates during thermal decomposition of carbohydrates in different foods and derives from Maillard reaction. Its control is required in products such as honey, because an excess of this compound has been associated with both low quality of food and unhealthy effects in consumers (Sanz,

Del Castillo, Corzo, & Olano, 2003; Morales, 2008). Given that we obtained elevated levels of this compound when both severe intensity of pressure and time were applied (600 MPa/ 6 min), we suggest discarding these specific conditions to preserve human milk.

3.4. Ketones

Four ketones have been isolated in the volatile profile of human milk. The total amount of ketones particularly increased in samples treated at 600 MPa for 6 min, particularly due to the high levels of 1,2-cyclopentanedione. So far, the origin of this compound remains unknown. In general, ketone levels were higher in control conditions than in samples treated with HPP technology for 6 min.

Ketones are compounds present naturally in dairy products. As a consequence of their typical odours and low perception thresholds, ketones contribute remarkably into the aroma of products that contain them. Floral and fruity notes are commonly linked with the presence of 2-nononone and 2-heptanone (Curioni & Bosset, 2002).

3.5. Furans and pyrans

The chromatographic signals corresponding with the compounds dihydro-4-hydroxy-2(3*H*)-furanone, and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one were not resolved correctly, since they overlapped with each other. For this reason, we decided to quantify together as 2dihydro-4-hydroxy-(3*H*)-furanone + 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (Table 1). Furans and pyrans showed low levels in both control samples and milk treated at 400 MPa for 3 min by HPP. Otherwise, HoP caused an increase (~ 20%) in their levels. However, it is important to note that treatments at 400 and 600 MPa for 6 min produced the most noticeable enhancement (~ 50%) of these

compounds in human milk.

In general, the formation of these compounds is associated with active carbohydrate degradation *via* Maillard reaction. Furthermore, it is important to note that thermal degradation of carbohydrates in food (Fagerson, 1969) takes place simultaneously with Maillard reaction. This thermal degradation produces conjugated compounds (reductones, furans derivatives, pyrans derivatives, cyclopentenones and other compounds) which are intermediate and/or resulting compounds that contribute to the aroma, taste and color of food. Due to the high levels of furans and pyrans in milk processed at 400 and 600 MPa for 6 min, it is possible that part of these complex reactions may be potentiated by some combinations of pressure and time, although other combinations did not affect the formation of these compounds.

Among furans and pyrans detected in human milk, a compound with a sulfur group has been isolated, tentatively identified as dihydro-3-(thioacetyl)-2(3*H*)-furanone. According to Steely (1994), sulfur compounds derive from thermal degradation of sulfur-containing amino acids of milk proteins. As shown in Table 1, HPP at 600 MPa for 6 min favoured the formation of dihydro-3-(thioacetyl)-2(3*H*)-furanone. Only this sulfur compound was detected in human milk, possibly because volatile sulfur compounds although possessing low threshold limit values are usually present at low levels in milk, providing a major challenge to detect and identify them.

Maltol and maltol propionate are compounds identified in treated samples but not in control samples. Their levels were significantly elevated in samples treated at 600 MPa for 6 min by HPP (Table 1). Maltol is formed by the cleavage of sugars, especially fructose, during its heating. It appears spontaneously in some processing food, such as

roasted malt (Chuyen, 1998; Mottram, 2007).

3.6. Alcohols

This chemical group was the least abundant group detected in the headspace of the milk samples. Its content remained constant after the different treatments applied, except for HPP at 600 MPa for 6 min, where the treated milk increased their content in alcohols ~30-fold over control. The vast majority of alcohols detected in the present study are originated from lipid oxidation, so their increase after this treatment would be in line with the high levels of other lipid-derived compounds such as aldehydes.

3.7 Inactivation of microorganisms

The study of Viazis et al., (2008) is the only one that studies in depth the microbiological effect of HPP at 400 MPa on breast milk. They demonstrated that HoP is an appropriate process for assuring microbial safety of human milk. Regarding treatments at 400 MPa, *Escherichia coli* and *Staphylococcus aureus* were the most pressure-resistant pathogens. They needed 30 min to reach 6–8 log₁₀ reductions. The rest of the pathogens tested, (*Listeria monocytogenes* and *Streptococcus Agalactiae*) were quite pressure-sensitive and were readily inactivated in shorter times (8-log₁₀ after two-four minutes). Taking into account these results, the treatments at 400 MPa for 3 or 6 min of this study would be insufficient to inactivate pressure-resistant microorganisms. However, our temperature of HPP was lower and the microbiological effect of our treatments is not known.

4. Conclusions

HPP at 600 MPa for 6 min increased the formation of aldehydes, furans and pyrans. All of these compounds are associated with an increase in lipid oxidation and with the

degradation of sugars and amino acids via Maillard reaction. The concentration of some volatile compounds is even higher in HPP treated samples (600 MPa/ 6 min) than in HoP treated milk samples. Therefore, the combination of severe pressure intensity and time would be discarded in milk banking. By contrast, HPP at 400 and 600 MPa for 3 min maintained volatile compounds at similar levels to those found in control milk samples. Treatment at 600 MPa/3 min is probably more suitable, as higher microbial inactivation would be expected under these conditions.

Taking everything into account, the use of HPP technology is an alternative to thermal pasteurisation applied to milk banking. However, it is important to highlight that not all HPP treatments are preferable to HoP. Moreover, before applying high-pressure technology in milk banking, it is important to study the effectiveness of this procedure at a microbiological level to ensure the equivalence of both technologies.

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Figure captions

Figure 1. Sum of volatile compounds ($\text{AAU} \times 10^3$) in each chemical group isolated from human milk processed by Holder pasteurisation (HoP) and high pressure processing (HPP; 400 MPa/3 min; 400 MPa/6 min; 600 MPa/3 min y 600 MPa/6 min).

Table 1. Volatile compounds (AAU $\times 10^3$) isolated of human milk processed by Holder Pasteurización (HoP) and high pressure processing (HPP; 400 MPa/3 min; 400 MPa/6 min; 600 MPa/3 min y 600 MPa/6 min).

	Rt (min) ^a	ID method _b	control	HoP	HPP 400 MPa		HPP 600 MPa		SE M	P-value
					3 min	6 min	3 min	6 min		
Aliphatic hydrocarbons										
Alkanes and cycloalkanes										
2,3,4-trimethylpentane-	17.3	RF	302a	0c	276ab	0c	199ab _c	59bc	31	0.001
3-ethyl-2-methylpentane	17.6	TI	453a	0d	412ab	97bcd	355ab _c	69cd	46	0.001
2,2,4-trimethylhexane-	19.0	RF	302a	0c	253ab	0c	155ab _c	66bc	29	0.002
1,2,4-trimethyl-cyclopentane	19.4	TI	1665a	0c	1461ab	418bc	788ab _c	688abc	146	0.003
octane	19.9	RF	121	19	96	42	61	100	11	0.072
2,4-dimethylhexane	21.0	TI	156ab	199a	132abc	37bc	140ab _c	0c	17	0.003
1,2,4-trimethylcyclopentane	24.1	TI	238a	0b	211a	118ab	126ab	64ab	21	0.008
(1,2-dimethylpropyl)-cyclopropane,	26.4	TI	93a	0b	89a	43ab	53ab	55ab	9	0.020
decane	28.6	RF	390ab	99b	544a	355ab	382ab	311ab	34	0.006
methylcyclooctane, -	30.1	TI	163a	0b	156a	121a	128a	173a	14	0.002

dodecane	35.3	RF	848	625	691	782	855	1067	54	0.319
tetradecane	41.1	RF	133a	0b	112a	0b	100a	0b	11	0.001
Terpenes										
α -pinene	26.4	RF	0b	40a	0b	0b	0b	0b	2	0.001
D-limonene	30.0	RF	728b	1250a	690b	671b	762b	591b	40	0.001
Aromatic hydrocarbons										
toluene	18.4	RF	149	50	160	81	122	58	15	0.181
<i>p</i> -xylene	23.5	RF	491ab	40b	612a	477ab	540ab	424ab	56	0.071
hexylbenzene, -	37.6	RF	49bc	33c	43bc	69b	50bc	105a	5	0.001
(2,4-cyclopentadien-1-ylidenemethyl)benzene,	41.4	TI	84a	35b	61a	58a	63a	74a	3	0.001
Carboxylic acids										
2,4-pentadienoic acid	25.9	TI	0b	182b	0b	872ab	72b	1640a	144	0.003
octanoic Acid	34.2	RF	1663a	799b	1600a	2038a	821b	272b	126	0.001
2-hydroxy-2-methyl-butanedioic acid,	39.7	TI	0c	1211b c	0c	1705b	192c	3729a	251	0.001
decanoic acid	40.0	RF	1664b	1150b	1453b	3891a	1156b	2503ab	226	0.001
dodecanoic acid	45.2	RF	920b	716b	811b	8970a	1088b	6833ab	836	0.002
Aldehydes										
hexanal	20.1	RF	282ab	172b	288ab	171b	240ab	479a	28	0.015
2-furaldehyde	21.8	RF	32	320	50	1550	105	2356	269	0.045
heptanal	24.8	RF	157	117	190	61	63	37	18	0.076

(<i>E</i>)-2-heptenal	27.1	TI	25	15	23	13	15	29	2	0.174
octanal + 2 <i>H</i> -pyran-2,6(3 <i>H</i>)-dione	28.8	RF	325b	452b	392b	607b	251b	1131a	66	0.001
2,5-furandicarboxaldehyde	31.6	TI	0c	183bc	0c	563ab	236bc	733a	61	0.001
5-(hydroxymethyl)-2-furancarboxaldehyde	36.4	RF	0b	1332b	246b	3790ab	480b	11616a	1084	0.005
(<i>E</i>)-2-decenal	37.4	TI	92b	45b	69b	179b	70b	715a	53	0.001

Ketones

3-heptanone	24.2	RF	127a	0b	140a	35ab	95ab	47ab	13	0.004
2-heptanone	24.3	RF	34	12	47	19	36	9	4	0.037
1,2-cyclopentanedione	26.1	TI	57b	412b	0b	1227b	126b	3011a	218	0.001
3-nonanone	31.8	TI	141ab	0d	160a	47cd	121ab	76bc	11	0.001

Furans and pyrans

2(5 <i>H</i>)-furanone (γ -Crotonolactone)	25.6	RF	84b	259b	56b	845ab	169b	1852a	167	0.007
dihydro-4-hydroxy-2(3 <i>H</i>)-furanone, + 2,3-dihydro-3,5-dihydroxy-6-methyl-4 <i>H</i> -pyran-4-one,	34.1	TI	0c	1448b	0c	3404a	360bc	325bc	227	0.001
3,5-dihydroxy-2-methyl-4 <i>H</i> -pyran-4-one	35.4	TI	0b	187b	0b	757ab	0b	1724a	150	0.001
dihydro-3-(thioacetyl)-2(3 <i>H</i>)-furanone,	38.9	TI	52b	196b	31b	288b	92b	1179a	90	0.001

2-methyl-3-(1-oxopropoxy)-4 <i>H</i> -pyran-4-one (maltol propionate)	31.9	TI	0b	535ab	12b	514ab	116ab	839a	85,4	0.010
3-hydroxy-2-methyl-4 <i>H</i> -pyran-4-one (maltol)	33.1	RF	0b	109b	2b	596ab	90b	1655a	156,4	0.005

Alcohols

2-ethyl-1-pentanol,	18.5	TI	62a	0c	27b	0c	0c	0c	4	0.001
2-furanmethanol	21.1	TI	0b	0b	0b	92b	0b	590a	47	0.001
1,2,3-propanetriol, 1-acetate (<i>1-Monoacetin</i>)	36.9	TI	0b	57b	0b	0b	0b	1542a	116	0.001
<i>p</i> -isopropenylphenol	38.6	TI	127a	70b	63bc	49bcd	43cd	27d	6	0.001
1,2,3-benzenetriol	40.9	TI	0b	0b	0b	377b	0b	1280a	99	0.001

^aRetention time ^bMethod of identification: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum; SEM, standard error media. a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's test, $p < 0.05$). Each value is expressed as mean of five repetitions.

- Thermal treatment caused variations in the profile of volatile compounds of breast milk
- Intense high pressure treatments produced strong modifications in the profile of volatile compounds of breast milk
- HPP at 400 and 600 MPa for 3 min maintained volatile compounds at similar levels to those found in control milk samples