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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.

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 pasteurisation, 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 et al., 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

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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. Materials 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 Maire 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 O_2/m^2/24 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; Contarini & Povolo, 2002; Frank, Owen, & Patterson, 2004; Jaillais, Bertrand, & Auger, 1999; Marsili, 1999; Shooter, Jayatissa, & Renner, 1999), 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,