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Volatile profile of human milk subjected to high-pressure thermal processing

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ABSTRACT

High pressure thermal (HPT) treatments consist of a combination of high pressure (500–900 MPa) and temperature (70–120 °C) over a short holding time. The rapid temperature increase during compression and temperature decrease in the product upon decompression could help to reduce the hardness of thermal effects encountered in conventional thermal technologies. The objective of this study was to evaluate the effect of HPT processing (300, 600 and 900 MPa combined with 50, 65 and 80 °C) on the profile of volatile compounds in breast milk. A total of 50 volatiles, belonging to 6 different chemical groups, were detected in milk samples. In general, HPT processing increased aldehydes, ketones, furans and pyrans, as well as alcohols, however, it did not modify the levels of carboxylic acids, and reduced the content of aliphatic hydrocarbons present in the non-treated human milk samples. HPT processing enhanced the total area content of volatile compounds derived from Maillard and lipid oxidation reactions, these changes being subjected to the intensity of pressure and temperature applied. Given that the levels of volatiles were significantly modified after the application of HPT processing, we can conclude that the range of the intensity of the treatments selected was not adequate to preserve the original profile of volatile compounds in breast milk.

1. Introduction

Breastfeeding is strongly encouraged since it improves host defenses, digestion and absorption of nutrients, gastrointestinal function, and neurodevelopment in infants. The unique composition of human milk confers specific protection against many pathogenic bacteria and viruses by virtue of its content of immune cells, cytokines and proteins like immunoglobulins, mainly secretory IgA, lactoferrin, lysozyme and lactoperoxidase (Ballard & Morrow, 2013). Therefore, donor milk storage in human milk banks may be an adequate substitute when the mother's own milk is unavailable for several reasons.

The main objective of human milk banks is to ensure nutritional and biological quality and safety of human milk donated. Generally, human milk banks apply low-temperature/long-time thermal pasteurization, a process in which donor milk is heated at 62.5 °C for 30 min, also known as Holder Pasteurization. This treatment eliminates potential pathogenic microorganisms and hence assures the microbial safety of milk donated. However, it is detrimental to the bioactivity of human milk. Indeed, pasteurization causes the loss of variable amounts of IgA, IgM, IgG, lactoferrin, some vitamins, and other milk components (Henderson, Fay, & Hamosh, 1998). Likewise, sterilization processing is not appropriate to preserve the quality of milk, in terms of its functional value, since it involves exposing food to a temperature generally exceeding 100 °C.

The High Pressure Processing (HPP) is a technique that can be applied to solid and liquid foods (usually 400–800 MPa; for short-term treatments 5–10 min) to provide microbiologically safe, nutritionally intact, and organoleptically high-quality products (Viazis, Farkas, & Jaykus, 2008). Although HPP inactivates pathogenic microorganisms, microbial spores cannot be inactivated by high pressure alone (Cheftel, 1995). At this respect, a more recent development in HPP is the use of moderate–high process temperatures (high pressure thermal; HPT) as this technology was found to allow more efficient spore inactivation. HPT processing involves simultaneous application of high pressure (100–900 MPa) and heating (50–120 °C) for a short holding time. Moreover, uniform compression during pressurization and adiabatic cooling upon decompression helps to reduce the negative effects of the conventional thermal treatment. Thus, it has been reported that HPT treatments provide better color, flavor and aroma retention compared to traditional thermally treated products (Gupta, Balasubramaniam, Schwartz, & Francis, 2010).

When tasting fresh breast milk, retronasal aroma is described by the respective mother as predominantly sweet, fatty, and soy bean-like, with slight buttery and cooked milk-like impressions (Spitzer & Buettner, 2010). Human milk has a characteristic and unique composition of volatile compounds which provide such odors. The character of

Abbreviations: AAU, arbitrary area units; GC, gas chromatography; HPP, High Pressure Processing; HPT, high pressure thermal; MS, mass spectrometry; UHT, ultra-high-temperature processed; SPME, solid-phase microextraction technique.

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the resulting aroma depends on a huge range of complicated mixtures of several compounds such as fatty acids, terpenoid substances, as well as saturated and unsaturated aldehydes and ketones, among others (Buettner, 2007; Spitzer & Buettner, 2009). Since, neonates are able to recognize specific smells with high reliability and to be greatly receptive for flavor learning (Mennella, Jagnow, & Beauchamp, 2001), the preservation of the original volatile compounds in human milk is a crucial concern.

The analysis of the changes in the volatile compounds of breast milk could serve as a general method to assess the effect of novel technologies, since modifications of these compounds could be the result of the development of multiple chemical reactions (oxidation, Maillard reaction, polymerization…) that takes place during milk processing.

Previous studies evaluated the effect of HPP on the volatile profile of human milk (Contador, Delgado, García-Parra, Garrido, & Ramírez, 2015). However, taking into account that there is no study about the effects of HPT processing on the volatile profile of human milk, the goal of this study was to evaluate the changes in the volatile compounds present in human milk after the application of different HPT treatments.

2. Material and methods

2.1. Human milk collection

Mature breast milk samples were collected from volunteer healthy mothers (n = 6; between 32 and 36 years old and between 3 and 26 months from parturition) from Maire Association (Association of Breastfeeding support of Badajoz, Spain) in July 2012. Each mother donated between 100 and 200 mL of milk that was collected during the previous day of the assay. Milk was kept in the refrigerator until the experiments were carried out during the next morning.

Before processing, all milk donations were mixed in 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 the processing. Milk aliquots (10 mL) were vacuum packed in polyethylene bags (9.3 mL O_2/m^2/24 h at 0 °C) for HPT processing. Control samples were also vacuum-packed in plastic bags. All bags have the same dimensions of 3.5 × 1.5 cm. Milk samples were protected from the light by using aluminum foil during sample manipulation. The time between mixing, packaging, processing and freezing the samples was less than 3 h. After processing, samples (processed and unprocessed samples) were stored at −80 °C (all at once) until analysis.

2.2. High pressure thermal (HPT) processing

A multi-vessel Resato unit (FPU-100-50, serial no. 14685/42798, Roden, Netherlands) was used for the application of the HPT processing; per vessel, one bag was processed at the time. Before the application of the treatments, the bags were inserted 1 min to equilibrate the sample temperature to the initial working temperature. Vessel volume was approximately 50 cm^3 (dimensions were approximately 3 cm of diameter and 8 cm of height). The machine uses ethylene glycol as pressure-transmitting medium, and was equipped with thermostatic jacket for temperature control. Initial temperature of glycol was 50, 65 and 80 °C and the pressures applied were 300, 600, 900 MPa for 1 min. This holding time was chosen because it was short enough to maintain a quasi-constant temperature of processing. This short holding time avoided the loss of heat during processing. Pressure build-up rate was 10 MPa s^{-1}. Temperature of milk during HPT treatment could not be measured during processing. We have measured the temperature changes of the pressurization medium (the thermocouple was in the middle of the cylinder vessel), so temperature of milk bags should be lower. However due to the low amount of milk in each bag, differences of temperature between the milk and the pressurization medium should not be very different.

The increases of pressure and temperature during the treatments applied are shown in Fig. 1. The multi-vessel Resato unit registered the temperature of all vessels during the whole process. These data were extrapolated into an excel file. Graphics show the temperature of the pressurization media and the pressure measured in the vessels was stable during the treatments applied to human milk. After processing, the bags containing the milk were submerged in cold water to rapidly reduce the breast milk temperature.

Four bags per treatment were processed, analyzed and compared with five control samples. So a total of 41 samples were studied.

2.3. Analysis of the volatile compounds in human milk

The identification and quantification of such compounds were effectuated by gas chromatography (GC) coupled to mass spectrometry (MS), with a prior step involving the isolation of the volatile fraction by solid-phase microextraction technique (SPME).

For the extraction, 8 g of human milk was placed in a 50 mL vial. The sample was stirred at 35 °C for 30 min to accelerate equilibrium of headspace volatile compounds between the milk matrix and the headspace. After that, volatile compounds were extracted by placing a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane SPME fiber (Supelco, Bellefonte, PA) into the vial and exposing it to the headspace at 35 °C for 30 min. 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. The temperature programmed was isothermal at 40 °C for 10 min, and then it was raised at 5 °C/min to 240 °C and held for 11 min. The GC–MS transferline temperature was at 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 compounds were identified by comparison with commercial reference compounds provided by Sigma-Aldrich (St. Louis, MO) and by comparison of their mass spectra with those contained in the NIST and Varian libraries. Volatile compounds were classified according to their most probable origin: lipid oxidation (L), Maillard reaction (M), and other reactions/sources (O). Due to the difficulties to classify all compounds, volatiles with unknown origin were marked as follows (−).

2.4. Statistical analysis

Data are expressed as mean values (n = 4 for processed milk and n = 5 for control milk) and standard error of the mean (SEM) of the numbers of determinations carried out. To compare the differences in the volatile profile of human milk after processing, statistical significance ($p < 0.05$) was calculated by one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. All these statistical treatments were performed using the SPSS program for Windows, version 17.0 (SPSS Inc., Chicago, IL).

3. Results and discussion

The purpose of this study was to evaluate the changes in the volatile compounds present in human milk after the application of different HPT treatments. For commercial sterilization of low-acid foods, such as milk, 12 log reductions of proteolytic *Clostridium botulinum* type A spores are required. A thermal treatment with a process value F0 of 3 min has been adopted as the minimum standard for a sterilization process. However, at industrial level generally an F0 of 5 min is applied. Until today, the limit to reach a sterilization effect is not clear. Vervoort et al. (2012) established that an initial temperature of 90 °C was required to result in a maximum temperature of 124.8 °C after pressure build-up till