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Ultrasonic inactivation of microorganisms: A compromise between lethal capacity and sensory quality of milk

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ABSTRACT

The aim of this study was to evaluate eight ultrasound (US) different combinations of amplitude (70 and 100%) and duration (50, 100, 200 and 300 s) for their lethal capacity against *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus* and *Debaryomyces hansenii*, the generation of volatile compounds (VC) and their impact on milk sensorial descriptors. The strongest treatment (100% × 300 s) led to a population reduction of 4.61, 2.75, 2.09 and 0.55 log for *D. hansenii*, *P. fluorescens*, *E. coli* and *S. aureus*, respectively, but caused milk sensorial deterioration. Four descriptors (metallic, burnt, rubbery and sharp) were correlated with treatment duration. Each of them, using stepwise regression analysis, was found to be associated with one VC (dodecanoic acid, $R^2 = 0.90$; octanoic acid, $R^2 = 0.82$; δ-dodecalactone, $R^2 = 0.81$ and decanoic acid methyl ester, $R^2 = 0.78$) that could be considered as a marker for milk sensorial deterioration caused by US.

Industrial relevance: In this study it is stated in what conditions ultrasounds (US) could be used as effective sanitation procedure without the production of off-flavours and what is the inactivation capacity toward *E. coli*, *P. fluorescens* and *D. hansenii* in that conditions. Moreover, our results indicate possible markers of milk sensory degradation caused by US treatments. These markers (dodecanoic acid, octanoic acid, δ-dodecalactone and decanoic acid methyl ester) could be used to check milk sensory quality after US treatment instead of using expensive and time consuming sensory tests.

1. Introduction

Among different technologies that are used to ensure the hygiene and to prolong the shelf life of milk, ultrasound (US) alone or US coupled with pressure or heat has been shown to inactivate many bacterial species (Piyasena, Mohareb, & McKellar, 2003) and to represent effective hygienic procedures (Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009), even though the sensitivity towards US has been reported to change within different microorganisms (Gao, Lewis, Ashokkumar, & Hemar, 2014). Moreover, US treatments have been reported to have both positive and negative effects on the characteristics of milk, on one side the improvement of its cheese-making properties, on the other the production of volatile compounds (VC) that result in off-flavours (Riener, Noci, Cronin, Morgan, & Lyng, 2009) that compromise the sensory quality (Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Marchesini et al., 2012). The effects of US, such as its lethal capacity versus various microorganisms, appeared to be dependent on the length of the treatment and the amplitude of the ultrasonic waves, which represent the intensity of the US treatment (Bermúdez-Aguirre et al., 2009; Cameron, McMaster, & Britz, 2008). Many authors achieved a 5 log-reduction only after treatments longer than 10 min as for *Escherichia coli* (Salleh-Mack & Roberts, 2007) and *Listeria monocytogenes* (D'Amico, Silk, Wu, & Guo, 2006; Gabriel, 2014). During US treatment, waves are generated and propagated within the liquid media, producing compressions and rarefactions. If the amplitude of the ultrasonic waves is sufficiently high, first the bubbles form in the liquid and then implode in a phenomenon known as cavitation (Villamiel & de Jong, 2000a). Cavitation causes shock waves that in turn create regions of high temperature and pressure, resulting in cell death (Cameron et al., 2008; Piyasena et al., 2003), disruption of the milk-fat globule membrane (Bermúdez-Aguirre, Mawson, & Barbosa-Cánovas, 2008) and the production of free radicals and other reactive species that can induce redox reactions in the medium (Riesz & Kondo, 1992).

This paper is the complementary part of a study (Marchesini et al., 2012) that investigated chemical and sensorial changes of milk submitted to several US treatments varying for intensity and duration. The aim of the present study was to evaluate the lethal capacity of US treatments against microorganisms belonging to Gram-negative, Gram-positive bacteria and yeasts at different combinations of intensity and time and to verify if the effectiveness of US treatments could be useless by the

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deterioration of the sensory quality of milk. Furthermore, the existence of a relationship between milk sensorial descriptors and VC was investigated to find useful chemical markers for the presence of off-flavours. The four microbes used in this study were chosen for different reasons. *E. coli* and *Pseudomonas fluorescens* are representatives of Gram-negative bacteria. Moreover, the first one is an indicator of faecal contamination (Cameron et al., 2008; Holsinger, Rajkowski, & Stabel, 1997) and the latter is one of the most important psychrophilic bacteria that are responsible for undesirable flavours in milk and other dairy products (Villamiel & de Jong, 2000b). *S. aureus* is a Gram-positive bacteria that is implicated in mastitis, very often in sub-clinical form, in dairy cows worldwide and that also causes foodborne diseases (Silva et al., 2013), whereas *Debaryomyces hansenii* was included because it is a yeast that has been isolated from milk and all types of cheese. The latter could be used in starter cultures to favour the onset of cheese flavours and sensorial characteristics (Breuer & Harms, 2006). *D. hansenii*, at our knowledge, was never tested before for its resistance to US in milk and represents a microorganism with a positive function, that should be preserved or even stimulated by treatment, especially where milk is intended for processing.

2. Material and methods

2.1. Experimental design

Eight combinations of amplitude (A: 70, 100%) and duration (D: 50, 100, 200 and 300 s) of US treatment were applied to determine their effects on the inactivation of *E. coli*, *S. aureus, P. fluorescens* and *D. hansenii* and the development of VC in raw milk; non-sonicated (D = 0 s) milk served as control. With the aim to determine the lethal effectiveness, the raw milk intended both for sonication and control was previously inoculated. The same combinations, with the exception of those with the longest duration (300 s), were applied to evaluate the effect of US on milk sensory properties, as reported in a previous paper (Marchesini et al., 2012).

2.2. Milk preparation

Full fat milk was obtained from a local dairy plant at three different times, one for each of the three independent samplings ($n = 3$). Immediately upon arrival at the dairy plant, raw milk was subdivided in two lots: raw milk (L1), intended for microbial inoculation and VC determination and pasteurised milk (L2), intended for the evaluation of the difference of sensorial attributes between sonicated and non-sonicated milk. The pasteurisation (74 °C for 15 s) was necessary to ensure, in a timely, the milk safety to the panellists.

2.3. Milk inoculation and US treatment

Milk samples from L1 were stored at 4 °C in the dark before being inoculated with the microorganisms (into 2 h from the collection). The challenges were arranged using the following references strains: *E. coli* (ATCC 8739), *S. aureus* (ATCC 6538), *P. fluorescens* (ATCC 13525) and *D. hansenii* var. *hansenii* (CBS 767). Cells in the stationary phase were diluted to a final concentration of 10^5 CFU mL^{-1} in the milk.

The US treatments were performed at a constant frequency of 24 kHz using a 400-W ultrasonic processor (UP400S, Hielscher Inc., Ringwood, NJ, USA). The processor was equipped with a 22 mm diameter probe. The average acoustic power transferred to the milk via US was calculated using the calorimetric method according to Mãnas et al. (2000) and was 160.4 J s^{-1}. The samples (100 mL) were treated in 150-mL glass beakers (Duran Group, Mainz, Germany), and the probe was positioned at a fixed depth inside the beaker.

In all of the experiments, the temperature of each sample was measured prior to and following the treatment using an infrared thermometer (830-T2, Testo spa, Settimo Milanese, Italy). Before starting with sonication, the beaker containing the sample was dipped in an ice bath to prevent the over-heating of the sample.

2.4. Microbiological analysis

The numbers of each microorganism were immediately evaluated after each US treatment and, at the same time, in control (non-sonicated) milk. Samples were plated and counted according to ISO (2001), ISO (1999), CCFRA (2003), and CCFRA (2007) protocols for *E. coli*, *S. aureus*, *P. fluorescens and D. hansenii,* respectively.

2.5. Sensory evaluation

The sensory evaluation procedure is reported by Marchesini et al. (2012). Briefly, a duo-trio test was conducted (ISO, 2004) in the morning and replicated in the afternoon of the day of collection. The panellists ($n = 29$) blindfold received in a random order two samples of milk from L2 (one control and one sample subjected to one of the US combinations) that had to be compared with a known control sample. The panellists had to choose the sample that was the most similar to the control and to describe the difference between the control and experimental sample using one or more of the following attributes: stuffy, rubbery, cardboardy, burnt, sharp, sour, toffee, boiled milk, metallic, plastic, sweet and bitter (Chouliara et al., 2010; Hougaard, Vestergaard, Varming, Bredie, & Hipsen, 2011; Karatapanis, Badeka, Riganakos, Savvaidis, & Kontominas, 2006).

2.6. SPME-GC/MS analysis of volatile compounds

Samples (10 mL) of raw milk (L1), from control (non-sonicated) and sonicated milk, were put in a 22-mL glass vial (20 × 72 mm, d × h) sealed with an aluminium cap. Solid-phase microextraction (SPME) was done using a 100-μm polydimethylsiloxane (PDMS) fibre (Supelco, Bellefonte, PA, USA). The sample was placed under stirring and brought to 40 °C in 5 min, after which the PDMS fibre was exposed to the vial's head space for 30 min.

GC/MS analysis of the volatile compounds adsorbed onto the SPME fibre was performed using a Shimadzu GC2010 gas chromatograph equipped with a QP2010 mass selective detector (Shimadzu Italia, Milano, Italy). A ZB5 column (5% phenyl-, 95% dimethylpolysiloxane, 60 m, 0.25-mm internal diameter, 0.25-μm film thickness; Phenomenex Inc., Torrance, CA, USA) and helium flowing at constant speed of 35 cm min^{-1} were used as capillary column and carrier gas, respectively. During the injection phase, the injector was operated in the on-column mode at 310 °C and for thermal desorption the SPME fibre was retained in the injector for 30 s. Initially the column was maintained at 35 °C for 4 min then heated to 300 °C at a rate of 5 °C min^{-1} and held at the final temperature for 10 min. The mass spectrometric (MS) conditions were: source at 220 °C; acquisition in the electron-impact (EI) mode (70 eV) with 2 scans s^{-1}; the mass/charge (m/z) range covered was 29–450. The temperature of the transfer line was constantly held at 220 °C. Peak identification was performed by comparing the retention times and the mass spectra of the eluted compounds to those in the NIST library (Standard Reference Data, NIST, Gaithersburg, MD, USA).

2.7. Statistical analysis

All of the statistical analyses were performed using SAS software (2010, release 9.3; SAS Institute Inc., Cary, NC, USA). The microbial data were subjected to statistical analysis as the log of the ratio between N_D (residual population, as CFU mL^{-1}) after the D^{th} sonication period (50, 100, 200 or 300 s) and N_0 (control population as CFU mL^{-1}, D = 0 s) within the A of the ultrasonic wave (70 vs. 100%). The data were preliminarily analysed as a complete randomised design using the REML variance component and the MIXED procedure with a CS