Food Control 47 (2015) 599–605

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Improved process for decontamination of whey by a continuous flow-through pulsed light system

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ARTICLE INFO

Article history:
Received 19 March 2014
Received in revised form
30 July 2014
Accepted 5 August 2014
Available online 14 August 2014

Keywords:
Food preservation
Light exposure
Mild processing temperatures
Listeria
Synergistic effect
Non-thermal process

ABSTRACT

The effect of a continuous flow-through pulsed light (PL) system on the inactivation of *Listeria innocua* was evaluated in different liquid substrates: distilled water, whey, diluted whey and skimmed whey. Reductions in *L. innocua* counts increased with number of pulses and total fluence. For similar total fluence, treatments consisting of a higher number of pulses but lower voltage were more effective in *L. innocua* inactivation due to a higher probability to expose cells to the incident light. Microbial inactivation by PL depended on the quantity of light transmitted in the range 230–290 nm by liquid substrates. Regardless of the limited effectiveness of PL for decontamination of whey, its shelf life during storage at 4 °C was extended by at least 7 days. In order to improve the antimicrobial effectiveness of PL, this treatment could be carried out at mild processing temperatures (60 °C), which was shown to exhibit a synergistic effect on the inactivation of *L. innocua*. This combined process appears as a promising technique to decontaminate complex substrates such as whey or to reduce the intensity of individual treatments to achieve the required level of microbial inactivation.

1. Introduction

Dairy industry produces significant amounts of liquid wastes, mostly whey obtained during the cheese making process. Whey disposal as untreated effluent creates huge environmental problems because of its high biological and chemical oxygen demand resulting from its great content of sugar, lactose and proteins (Mahmoud & Ghaly, 2004; Siso, 1996). In order to reduce environmental impacts and increase economic competitiveness of the dairy industry, a wide variety of applications have been developed for the valorization of dairy processing by-products as a source of high added-value components. Whey proteins (mainly β-lactoglobulin and β-lactalbumin) can be used, for example, as nutritional ingredients in different food products such as infant foods, protein fortified beverages or nutritional supplements (Kosikowski, 1979; Madureira, Tavares, Gomes, Pintado, & Malcata, 2010). Moreover, whey proteins have also excellent functional and technological properties for the manufacture of transformed food products (Kosikowski, 1979; Morr & Foegeding, 1990).

However, whey and other dairy processing by-products may provide an excellent environment for the growth and proliferation of a wide range of microorganisms (Chandan, 2011), being *Listeria monocytogenes* one of the major safety concerns in dairy industry (Kozak, Balmer, Byrne, & Fisher, 1996). Although pasteurization has been traditionally used to reduce microbial load present in whey, high temperature processes are known to denaturize whey proteins (Anema & Li, 2003) causing substantial changes in their nutritional, organoleptic and/or technological properties. Therefore, major efforts have been made to develop non-thermal technologies which can prevent these adverse thermal effects and produce safe food products.

Pulsed light (PL) appears as a promising alternative to conventional thermal processes for whey decontamination. This technology consists of a successive repetition of short duration and high power flashes of broadband emission light (190–1000 nm) with approximately 40% of the emitted light corresponding to the UV region. PL has been successfully tested for the inactivation of pathogenic and spoilage microorganisms in liquid dairy foods (Krishnamurthy, Demirci, & Irudayaraj, 2007; Miller, Sauer, & Moraru, 2012; Palgan et al., 2011b; Smith, Lagunas-Solar, & Cullor, 2002). Moreover, PL can induce conformational changes in whey proteins such as β-lactoglobulin, enhancing its absorption rate at the air/water interface and forming highly elastic interfaces (Fernández et al., 2012). In consequence, PL seems to be an effective method for inactivating microorganisms present in whey without

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http://dx.doi.org/10.1016/j.foodcont.2014.08.006
0956-7135/

compromising product quality and improving, at the same time, their foaming properties.

However, whey decontamination by PL could be limited by the restricted penetration of the light into the fluid, as it has been shown for other liquid food products (Artíguez, Arboleya, & Martínez de Marañón, 2012; Hsu & Moraru, 2011). In addition, as previously suggested by Gómez-López, Devlieghere, Bonduelle, and Debevere (2005b), the high amount of fats and proteins contained in whey could absorb a part of the wavelengths of the incident light and decrease the antimicrobial effectiveness of PL treatment. Due to the difficulty in decontaminating complex food matrices, the antimicrobial effectiveness of PL process could be improved by combination with other preservation methods, such as pulsed electric fields (Caminiti et al., 2011; Palgan et al., 2011a; Muñoz et al., 2012), thermosonication (Muñoz, Caminiti, et al., 2011; Muñoz, Palgan, et al., 2011), manothermosonication (Palgan et al., 2011a), ultrasound or sublethal concentrations of nisin or lactic acid (Muñoz et al., 2012). In this way, moderate processing temperatures could enhance PL treatment effectiveness, as described for other decontamination technologies which were combined with moderate thermal treatments (Bazhal, Ngadi, Raghavan, & Smith, 2006; Gayán, Monfort, Álvarez, & Condón, 2011; Geveke, 2008; Geveke & Brunkhorst, 2008; Heinz, Toepfl, & Knorr, 2003). However, the impact of the combination of milder thermal treatments with PL processing in a continuous flow-through system on microbial inactivation has not been assessed yet.

Despite the relevance of the composition, turbidity or opacity of liquid products on the effectiveness of PL for microbial inactivation, there is only one study reporting different levels of microbial inactivation by a continuous flow-through PL system in different liquid substrates (Pataro et al., 2011). Therefore, the impact of product characteristics on microbial inactivation by PL in a continuous flow-through system is not fully elucidated.

Therefore, the main objective of this work was to evaluate the antimicrobial effectiveness of PL in a continuous flow-through system. In order to determine the influence of product related factors, the effect of PL for microbial inactivation was assessed on different liquid substrates such as distilled water, whey, diluted whey or skimmed whey. In addition, PL was applied at different processing temperatures to define a novel combined process suitable for decontaminating non-transparent fluids such as whey. For those purposes, *Listeria innocua* was used as a surrogate for *L. monocytogenes*, pathogen associated with foodborne infections caused by the consumption of processed dairy products (Goulet, Hedberg, Le Monnier, & de Valk, 2008; Todd, 2011). Furthermore, this bacterium has been reported as one of the most resistant microorganisms to PL (Gómez-López, Devlieghere, Bonduelle, & Debevere, 2005a; Lasagabaster & Martínez de Marañón, 2012).

2. Materials and methods

2.1. Microorganism and growth conditions

The strain of *L. innocua* CECT 910 was specifically chosen because it was proposed as a good surrogate for *L. monocytogenes* in PL technology validation, after showing a similar resistance to PL than six *L. monocytogenes* strains, of five different serotypes, isolated from food products and/or food processing environments (Lasagabaster & Martínez de Marañón, 2012). This strain (*L. innocua* CECT 910) was preserved at −80 °C in Brain Heart Infusion (BHI; Pronadisa, Madrid, Spain) supplemented with 20% glycerol. Prior to each experiment, thawed stock cultures (200 μL) were transferred to 10 mL of BHI and pre-cultured at 37 °C for 24 h. Each bacterial strain was then inoculated at 10^3 CFU/mL in BHI tubes and cultured at 37 °C for 24 h until early stationary growth phase (10^9 CFU/mL). Cells were harvested by centrifugation (5804R centrifuge, Eppendorf AG, Hamburg, Germany) at 10,000 × *g* for 15 min at 4 °C, washed twice with Potassium Phosphate Buffered Saline (KPBS; 0.01 M K_2HPO_4, 0.01 M KH_2PO_4, 0.15 M NaCl; pH: 6.7) and finally resuspended in this buffer at a cell density of approximately 10^9 CFU/mL.

2.2. Samples inoculation

The effect of PL treatment on microbial inactivation was evaluated in different liquid substrates: distilled water, whey, diluted whey and skimmed whey. Whey was collected from a local farmer and stored at 4 °C until used for less than 24 h. Whey samples were submitted to different degrees of dilution (3/4, 1/2, 1/4, 1/8) in distilled water. Skimmed whey was prepared using an electric skimmer (Arroyo Laboratories, Santander, Spain).

Samples containing 1.5 L of corresponding liquid substrate were inoculated immediately before PL treatments with adequate volumes of *L. innocua* suspension (prepared as described above) in order to achieve approximately 10^7 CFU/mL.

2.3. Transmittance spectrum of the liquids substrates

The spectral transmittance of all liquid substrates was determined each 1 nm from 190 to 1000 nm with a Genesys 6 UV/VIS spectrophotometer (Thermo Spectronic, Rochester, NY, USA). All measurements were performed in Quartz Suprasil cuvettes of 2 mm of pathlength (Hellma, Mülheim, Germany), thickness similar to the PL reactor (see Section 2.5). Distilled water was used as the blank. At least three measurements per liquid sample were performed. The integrated area under the transmittance spectrum of each protein solutions within the range 230–290 nm, defined as arbitrary units, was determined using KaleidaGraph (Synergy Software, Reading, PA, USA).

2.4. Heating of water and whey samples

To evaluate the effect of PL at moderate processing temperatures on *L. innocua* inactivation, liquid samples were preheated by immersing in a hot water bath (Precisterm, J. P. Selecta S. A., Barcelona, Spain) until the required initial temperature was reached and immediately after, subjected to PL treatment. Controls were performed to determine the effect of temperature individually. For that purpose, liquid samples were heated to the desired temperature and pumped through the PL system with the lamps turned off.

2.5. Pulsed light treatment

PL treatments were performed by a dynamic flow-through pilot unit (Maria PUD system, Claranor, Manosque, France) composed by an electronics bay with a control, a loading and a cooling unit; and a hydraulic system with reactors, a launch tank and a volumetric circulating pump. The spectrum of light emitted by this system comprises wavelengths from 190 to 1000 nm, with a considerable amount of light in the short-wave UV spectrum, being the duration of each pulse 0.3 ms.

Liquid samples were pumped through a reactor of 2.15 mm of thickness and exposed to different number of pulses at voltage input of 1000 or 3000 V, being the corresponding pulse fluence 0.11 and 1.1 J/cm^2, respectively. Total fluence (H), or amount of photons striking on the sample per area unit, was calculated for each different treatment condition by multiplying pulse fluence (H_0) by the number of emitted light pulses (n) [$H = H_0 \times n$]. The effect of total fluence up to a maximum of 11 J/cm^2 on *L. innocua* inactivation