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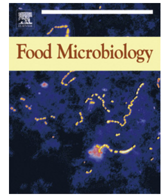

Light based technologies for microbial inactivation of liquids, bead surfaces and powdered infant formula

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ABSTRACT

This study evaluates the potential of continuous wave Ultraviolet C light (UV-C) and broad-spectrum intense pulsed light (in this study referred to as High Intensity Light Pulses, HILP) for the inactivation of pathogens of public concern in powdered infant formula (PIF) producers. To achieve this goal a sequential set of experiments were performed, firstly in clear liquid media, secondly on the surface of spherical beads under agitation and, finally in PIF. *L. innocua* was the most sensitive microorganism to both technologies under all conditions studied with reductions exceeding 4 $\log_{10}$ cycles in PIF. In the clear liquid medium, the maximum tolerance to light was observed for *C. sakazakii* against UV-C light and for *B. subtilis* spores against HILP, with a fluence of approximately 17 mJ/cm^2 required for a 1 $\log_{10}$ cycle inactivation (*D* value) of each species. In PIF it was possible to inactivate >99% of the vegetative cell populations by HILP with a fluence of 199 mJ/cm^2 and of *B. subtilis* spores by doubling the fluence. By contrast, for UV-C treatments a fluence of 2853 mJ/cm^2 was needed for 99.9% reduction of *C. sakazakii*, which was the most light-resistant microorganism to UV-C. Results here obtained clearly show the potential for light-based interventions to improve PIF microbiological safety.

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1. Introduction

Powdered infant formula (PIF) is the most widespread and established alternative to the breastfeeding of the newborn and is characterised by a rapidly growing market which was estimated to be worth US$41 billion globally in 2013 and is expected to reach US$27 billion by 2017 in China alone (Kent, 2015). As the sole source of nutrition for neonates and babies, PIF must be safe and nutritionally adequate for their healthy growth and development, thus its safety and quality are of upmost importance for manufacturers. In Europe PIF is produced under strict microbiological standards specified in the EU regulation (EC) No. 2073/2005 (EU, 2005) as newborn and infants are particularly susceptible to foodborne diseases with potentially severe sequelae associated with infection. However, studies on the presence of harmful bacteria in different locations within the PIF processing plant and in unopened packages have conclusively shown that PIF is not a sterile product (Mullane et al., 2007; Reich et al., 2010). Although they are generally present at low levels in PIF, these bacteria can potentially multiply to hazardous levels once the powder is rehydrated prior to consumption, particularly if recommended procedures are not followed. In fact, cases of diarrhoea, meningitis, brain damage and even death in babies and infants have been linked to PIF feeding (FAO/WHO, 2006). A number of bacterial pathogens of concern in PIF have been identified by WHO and FAO including *Cronobacter sakazakii*, *Salmonella* enterica, *Listeria monocytogenes* and *Bacillus cereus*, with *Cronobacter* and *Salmonella* classed as pathogens with 'clear evidence of causality' posing the highest risk of illness for newborn (FAO/WHO, 2006). As a result, preventing contaminated infant formula from entering the supply chain is of paramount importance for safeguarding the health of babies and places the onus of responsibility on PIF manufacturers to ensure the highest standards of safety are consistently delivered to protect public health.

As indicated, despite the preservation steps that the product undergoes during production, recontamination can occur at later stages of manufacturing and therefore the application of additional

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non-thermal hurdles as preventative measures would be desirable in this regard. According to the EU regulation on novel foods ((EU) No. 2015/2283) '*New technologies and innovations in food production should be encouraged as they could reduce the environmental impact of food production, enhance food security and bring benefits to consumers as long as the high level of consumer protection is ensured*' (EU, 2015). Light based technologies such as continuous wave Ultraviolet C light (UV-C, 254 nm) and broad-spectrum intense pulsed light (in this study referred to as High Intensity Light Pulses, HILP, 200–1100 nm) are potential hurdles that could be applied as a post-production process in dry infant formula manufacture. Traditionally, UV-C light irradiation has been used for air, drinking water, wastewater and surface decontamination though the food industry has recently shown increasing interest in this technology for the hygienization of liquid foods and the surfaces of solid foods. By contrast, HILP is a novel technology, which is a modified and, it is claimed, improved method of delivering UV-C to bodies, with a great potential for food decontamination (Gómez-López et al., 2007; Oms-Oliu et al., 2010). Both offer multiple advantages for food processing such as the effective inactivation of a broad range of spoilage and pathogenic microorganisms, minimal loss of the nutritional and sensorial quality of foods, no known toxic effects or residues formed during treatment and low energy requirements compared to other thermal and non-thermal processes (Gómez-López et al., 2007; Oms-Oliu et al., 2010). In fact, there is sufficient evidence in the literature that demonstrates that both UV-C light and HILP hold considerable promise as a viable alternative to thermal pasteurization/sterilization for a range of liquid foods and ingredients. However, their application in powdered or granulated foods with low a_w has scarcely been studied (Ha and Kang, 2014; Condón-Abanto et al., 2016).

The objectives of the current study were to evaluate the potential of UV-C light and HILP technologies for the inactivation of a number of PIF-related pathogens firstly, in liquid media, secondly, on the surface of spherical beads and, finally, in powdered infant formula.

2. Materials and methods

2.1. Microorganisms and growth conditions

Strains of *Cronobacter sakazakii* ATCC 29544, *Salmonella* Typhimurium NCTC 13348 (DT104) and *Listeria innocua* ATCC 33090 (as a surrogate for *L. monocytogenes*) were purchased from the National Collection of Type Cultures (NCTC, Public Health England, UK) and used in this study. These species were selected according to the current European legislation on microbiological criteria for foodstuffs ((EC) No. 2073/2005) (EU, 2005) for dried infant formulae intended for infants below six months (*Cronobacter* spp. and *Salmonella* risk, limit: absence in 10 and 25 g during shelf-life, respectively) and ready-to-eat foods intended for infants (*L. monocytogenes* risk, limit: absence in 25 g during shelf-life). Culture stocks were stored in cryovials at −20 °C. Microorganisms were resuscitated by inoculating a loopful of cryovial content into a test tube containing 5 mL of Tryptone Soya Broth (CM0129, Oxoid, England) supplemented with 0.6% Yeast Extract (LP0021, Oxoid) (TSBYE) and grown overnight at 37 °C. A loopful of this preculture was then streaked onto Tryptone Soya Agar (CM0131, Oxoid) plates enriched with 0.6% Yeast Extract (Oxoid) (TSAYE) and incubated for 24 h at 37 °C. Cultures were obtained by transferring a single colony from the TSAYE plate to 100 mL Duran bottles containing 60 mL of TSBYE and grown for 24 h at 37 °C. Stationary phase cultures with a final concentration of approximately 5×10^8–1×10^9 CFU/mL were centrifuged for 15 min at 5100 rpm (4216×*g*) (Laboratory Centrifuge 4 K 15, Sigma Zentrifugen GmbH, Germany) and the pellet washed and centrifuged twice in Maximum Recovery Diluent (MRD, pH 7.0, absorption coefficient ≈0.7 cm^{-1}) (CM0733, Oxoid) to be further re-suspended in 3 mL MRD for the light treatments in liquid or directly used for the inoculation of glass beads. In addition, commercially available vials of *Bacillus subtilis* DSM 618 endospores suspended in distilled water (ref. 110649 spore suspension, Merck KGaA, Germany) were also used in this study as a control for bacterial spores since such spores remain a significant challenge to PIF manufacture and can withstand thermal pasteurization. Initial endospore concentrations in the vials ranged from 8×10^6 to 5×10^7 CFU/mL.

2.2. Bead inoculation

Soda lime glass beads (ref. 4901002, Paul Marienfeld GmbH & Co. K.G., Germany) were used as a model dry particle food system for their uniform spherical shape and solid nature to study the microbial inactivation on a solid dry surface. Soda lime glass beads are not UV-transparent with a 2 mm diameter (⌀) and an approximate weight of 0.012 g. For the inoculation of the beads with bacteria and further exposure to light treatments, 6 g of sterile glass beads were vortex-mixed with the pellet obtained from either 25 mL of *C. sakazakii* or *L. innocua* stationary-phase cultures or 10 mL of a *B. subtilis* spore suspension and then left overnight for drying in a biological safety cabinet (Advanced Bio Safety Cabinet Class 2, Astec Microflow, UK). After drying, inoculated beads were exposed to UV-C and HILP treatments under agitation as explained in section 2.5.

2.3. PIF inoculation

In this study the procedure for the microbial inoculation of PIF (Similac Gain, AI-Q plus, Abbott Laboratories, USA) required the use of already inoculated glass beads and was a modification of the method described by Dhaval et al. (2010). For this, 15 g of dried glass beads previously inoculated with a 50 mL culture pellet of either *C. sakazakii*, *S.* Typhimurium, *L. innocua* or *B. subtilis* spores were mixed with 14 g of PIF and the mixture was vortexed for 3 min, and manually shaken for a further 2 min. A sterile metal strainer (1 mm mesh size) was used for the separation of the beads from the PIF. The inoculated PIF was then stored in sterile polycarbonate containers (Sarstedt Ltd., Ireland) in the dark at ambient temperature (≈18 °C). Final microbial concentrations in PIF were approximately 10^8 CFU/g for *C. sakazakii* and *S.* Typhimurium and 10^6 CFU/g for *L. innocua* and *B. subtilis* spores. Following the same procedure, PIF was also inoculated with a low level of *C. sakazakii* (approximately 10^4 CFU/g) to test whether the microbial inactivation was related to the initial microbial numbers.

2.4. UV-C and HILP equipment, energy and temperature measurements

A laboratory-scale UV-C light treatment chamber was custom-made and consisted of a stainless steel chamber (79 × 39 × 34.5 cm, length × width × height) containing 4 × 95 W low-pressure mercury lamps of 50 cm length mounted 5 cm from each other (ref. 892095 IP 65, Baro Applied Technology Ltd., Manchester, UK) emitting UV-C light continuously at 253.7 nm (monochromatic) (Haughton et al., 2011). The HILP unit used was a bench top Steri-Pulse XL3000 Pulsed Light Sterilization system (Xenon Corporation, MA, US) which consisted of a stainless steel chamber with a Xenon flash-lamp isolated with a quartz window mounted on top. The xenon lamp generates broad range (200–1100 nm, polychromatic) high energy pulses of light of 360 μs width at a fixed frequency (3 Hz).

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