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Pulsed light inactivation of *Bacillus subtilis* vegetative cells in suspensions and spices

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ABSTRACT

The aim of our study was to assess the impact of a pulsed light (PL) technology on *Bacillus subtilis* grown on two model matrixes, through bacterial inactivation and cell integrity. First, the effect of PL treatment on *B. subtilis* vegetative cells in liquid state was evaluated. We found that PL treatment leads to 8 log reduction of *B. subtilis* vegetative cells while bacterial morphology was not affected. Second, spices, among which ground caraway, ground red pepper and ground black pepper were artificially inoculated with a known concentration of *B. subtilis* in vegetative state and then exposed to PL. In this case, PL treatment leads to a bacterial reduction of about 1 log and serious damage of the microorganisms parietal structure occurred. To conclude, this work highlights that pulsed light has a potential for inactivation of *B. subtilis* in both, liquid and dry state but through different mechanisms.

1. Introduction

Nowadays, commercial and legal requirements regarding the safety, quality and storage of food products are focused on the development and improvement of decontamination methods. For pasteurising spices, the most often used decontamination techniques are thermal processing (dry heat or steam), irradiation, fumigation and vacuum gassing. However, it has been reported that these common decontamination techniques cause colour loss, vitamin destruction, flavour changes, or essential oil loss in herbs and spices (Gould, 1964; Grahl & Markl, 1996; Vajdi & Pereira, 1973). Microbial reduction by using Pulsed Light (PL) is gaining researchers attention thanks to the reduction in energy consumption compared to thermal processes (Barbosa-Canovas, Gongora-Nieto, & Swanson, 1998) and because it does not involve the use of products that cause environmental pollution. PL technology relies on very short, high-power pulses of broad-spectrum light, typically emitted by xenon lamps, to destroy bacteria (both vegetative cells and spores), yeasts, moulds and even viruses (Woodling & Moraru, 2006). Depending on the energy delivered through each flash, the physical characteristics of the pilot, the distance between the lamps and the contaminated matrix, the targeted microorganism and even the nature of the contaminated matrix itself, PL has been reported to result in a 0.5 to 8 log bacterial reduction (Hsu & Moraru, 2011). The germicidal action of PL has been attributed to the combination of the rich broad-spectrum UV content (responsible for the formation of lethal thymine dimers on bacterial DNA), and to the localized elevated temperature due to the UVs and IR radiations leading to bacterial disruption (Dunn, Clark, & Ott, 1995; Wekhof (2000); Takeshita et al., 2003). Although the exact mechanisms responsible for cell death are still not yet fully elucidated, many studies indicate a major contribution of the lower UV portion (250–260 nm) of the spectrum in bacterial inactivation (Uesugi & Moraru, 2009).

Dried spices are widely used in food mixtures, salad dressings, instant soups, frozen pizza and many other convenience food. Despite their contribution to taste, colour and aroma in food, these powders are known to be highly contaminated by various microorganisms, among which *Bacillus* spp., *Salmonella*, *Escherichia coli*, *Clostridium perfringens*, and aflatoxigenic *Aspergillus* (USFDA, 2002). This high contamination level is caused by poor sanitary conditions during growing, processing or storage. *Bacillus subtilis* is one of the most frequently encountered spore-forming bacteria in spices. The spices contamination frequently reaches 8 log CFU/g (Keith, 1998; McKee, 1995). *B subtilis* is a food-poisoning bacteria, known as a human non-pathogen but occasionally, leading to characteristic toxi-infections causing severe vomiting, abdominal cramps and diarrhoea. Most of the outbreaks reported have been connected with mayonnaise, pickled onion, canned bean salad and custard powder consumption for example (Gilbert, Turnbull, Parry, & Kramer, 1981;

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Kramer & Gilbert, 1989; Kramer, Turnbull, Munshi, & Gilbert, 1982). *B. subtilis* are resistant to several food processing steps and able to survive the sterilization process. It is therefore necessary to inactivate vegetative cells and spores in order to ensure food safety.

PL treatment is effective for the inactivation of bacteria (vegetative cells and spores) on surfaces, packaging and recently in food products (meat, bread, vegetables, and fruits) (Sauer & Moraru, 2009; Uesugi & Moraru, 2009) but few data are available on PL treatment of powdered/granulated food ingredients (Fine & Gervais, 2004). In the past few years, some studies (Fine & Gervais, 2004; Keith, Harris, Hudson, & Griffiths, 1997; Staack, Ahrné, Borch, & Knorr, 2008; Takeshita et al., 2003) have been dedicated to the investigation of microbial decontamination of food powders by using different non-thermal processing technologies. Keith et al. (1997) have investigated the effectiveness of pulsed electric fields (PEF) as another alternative method of reducing microbial levels in spices. These authors have studied PEF parameters (e.g. pulse period, pulse number, pulse shape) and successive treatments applied for the reduction of microbial levels in basil, dill and onion powder. The effect of infrared heating on the microbial decontamination of paprika powder has been studied by Staack et al. (2008) and at a_w 0.8, a reduction of 1–2 log CFU/g was obtained for a treatment of 11 and 5 kW/m^2. Despite this specific contribution, there is still a lack of knowledge in the literature, concerning the decontamination of powdered food by using PL treatment. The aim of the present work is thus to evaluate the effectiveness of a PL treatment on the inactivation of *B. subtilis* in spices.

2. Materials and methods

2.1. Bacterial strain and growth conditions

B. subtilis strain (ATCC 6633, Institut Pasteur, France) was grown at 30 °C for 24 h in M17 medium (Merck, Germany) supplemented with 0.5% glucose, under vigorous shaking (180 rpm). Cultures were centrifuged once at 10,000 *g* for 20 min at 20 °C and diluted in 0.9% saline solution in order to reach an OD_{580nm} of 0.8. Vegetative cells (10 mL) were exposed to PL as described in Section 2.3. All experiments were repeated at least three times.

2.2. Inoculation of spices with B. subtilis vegetative cells

Spices (3 g of ground caraway, ground red pepper and ground black pepper) (Colin Palc, France) were added to 25 mL of *B. subtilis* culture grown to stationary phase (OD_{580nm} of 0.8), then submitted to a gentile agitation (180 rpm) at 30 °C for 4 h. After centrifugation at 10,000 *g* for 20 min at 20 °C, the inoculated spices were spread on plates in a thin layer and dried in sterile conditions at room temperature for 15 h. Each spice was recuperated in a stomacher bag and mixed for 1 min by using a stomacher lab machine (Smasher AES, France) in order to obtain spices powder as before inoculation. PL treatment was then carried out as described in Section 2.3. The control and flashed samples were resuspended in peptone salt medium (PS) (Biokar Diagnostics, France) and shaken for 2 min to reconstitute the cellular suspension. Decimal dilutions were spread on PCA medium (AES, France). Plates were incubated for 24 h at 30 °C and CFU was counted. All samples were studied in triplicate.

2.3. Pulsed light treatments

The PL experimental set-up provided by Claranor (France) is composed of a power supply unit and a treatment chamber which contains four cylindrical xenon lamps (Massier et al., 2011). This experimental device generates a broad spectrum of PL ranging from 200 to 1100 nm with pulse duration of 300 μs. In this work, we chose to study the samples in two different conditions (0.6 J cm^{-2}/flash for the bacterial suspensions and 1 J cm^{-2}/flash for spices because of the strong risk of shadowing possibility occurring during the treatment).

Bacterial suspensions were treated in a quartz tank at 3000 V, 1 Hz with 1, 2, 4, 6, 8, and 10 pulses of light (F) using a 4 lamp configuration and the fluency was of 0.6 J cm^{-2}/flash (Fig. 1a and Table 1). Inoculated and control spices were put in a plastic circular box and then treated under circular agitation at 3000 V, 1 Hz with 10 F by using a 3 lamp configuration leading to a fluency of 1 J cm^{-2}/flash (Fig. 1b and Table 1). After exposure to PL, treated and untreated samples were analysed immediately.

2.4. Bacterial viability

Cell viability was first quantified by serial dilutions in 0.9% NaCl solution, and subsequently by plating on M17 medium (Merck, Germany). Plates were numerated after incubation for 24 h at 30 °C. Cell viability was also evaluated by the qDVC method (Yokomaku, Yamaguchi, & Nasu, 2000) as described below. The *B. subtilis* strain in stationary phase was incubated in two fold-diluted Luria-Bertani (LB) broth containing 10 μl novobiocin (4 mg/ml) and glycine (2% w/v) for 140 min at 37 °C in the dark (qDVC incubation). Selective lysis of viable cells in samples after qDVC incubation was carried out by using lysozyme (1 mg/ml) at 4 °C for 15 min. Bacterial cells remaining after the lysis made with lysozyme were stained with a 1/10,000 dilution of a stock solution of Syto X Green nucleic acid stain (Invitrogen, UK) and 5 min after the colouration step, 3 μl were deposited on a sterile microscope glass slide. Cell images were

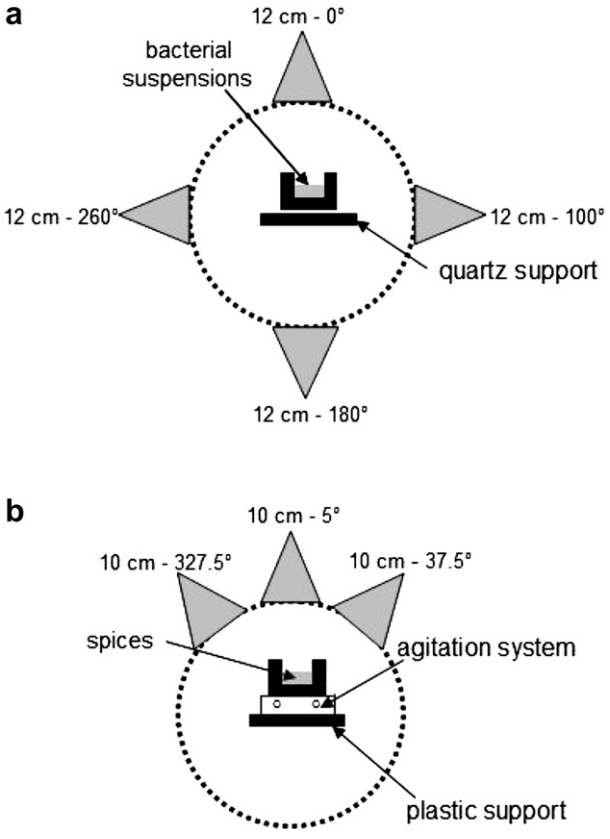

Fig. 1. Schematic illustration of the PL experimental set-up (Claranor): (a) a four lamps configuration, applied for the bacterial suspension treatment and (b) a three lamps configuration, employed for the spices decontamination.