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ORIGINAL ARTICLE

Inactivation of *Alicyclobacillus acidoterrestris* ATCC 49025 spores in apple juice by pulsed light. Influence of initial contamination and required reduction levels

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KEYWORDS
Pulsed light;
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Weibull model;
Biphasic model;
Inoculum size

Abstract The purpose of this study was to analyze the response of different initial contamination levels of *Alicyclobacillus acidoterrestris* ATCC 49025 spores in apple juice as affected by pulsed light treatment (PL, batch mode, xenon lamp, 3 pulses/s, 0–71.6 J/cm^2). Biphasic and Weibull frequency distribution models were used to characterize the relationship between inoculum size and treatment time with the reductions achieved after PL exposure. Additionally, a second order polynomial model was computed to relate required PL processing time to inoculum size and requested log reductions. PL treatment caused up to 3.0–3.5 log reductions, depending on the initial inoculum size. Inactivation curves corresponding to PL-treated samples were adequately characterized by both Weibull and biphasic models (R^2_{adj} 94–96%), and revealed that lower initial inoculum sizes were associated with higher inactivation rates. According to the polynomial model, the predicted time for PL treatment increased exponentially with inoculum size.

PALABRAS CLAVE
Luz pulsada;
Jugo de manzana;
Alicyclobacillus acidoterrestris;
Modelo de Weibull;
Modelo bifásico;
Tamaño de inóculo

Inactivación de esporas de *Alicyclobacillus acidoterrestris* ATCC 49025 por luz pulsada en jugo de manzana. Influencia de la contaminación inicial y de las reducciones requeridas

Resumen El objetivo del presente trabajo fue evaluar la influencia de la concentración de esporas de *Alicyclobacillus acidoterrestris* ATCC 49025 en la respuesta de inactivación por acción de la luz pulsada (modo estanco, lámpara de xenón, 3 pulsos/s, 0–71,6 J/cm^2) en jugo de manzana comercial. Para caracterizar la relación existente entre la concentración de esporas y el tiempo de tratamiento con las reducciones logarítmicas alcanzadas luego de la exposición

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a la luz pulsada (LP), se aplicaron 2 modelos: el de Weibull y el bifásico. Adicionalmente, se estimó la relación entre el tiempo de tratamiento con LP y la concentración inicial de inóculo en el jugo con las reducciones logarítmicas logradas mediante regresión múltiple y la metodología de superficie de respuesta (MSR). La inactivación por LP provocó entre 3 y 3,5 reducciones logarítmicas, según la concentración inicial de esporas. Las curvas de inactivación fueron adecuadamente caracterizadas por los modelos matemáticos propuestos ($R^2_{ajustado} = 94–96\%$). El análisis por MSR permitió predecir un aumento exponencial del tiempo de tratamiento requerido conforme se incrementa el nivel de contaminación inicial.

Introduction

Heat pasteurization is the most commonly used technique for fruit processing as it ensures microbial safety and shelf life of juices. Nevertheless, it is well-known that traditional thermal processes cause significant damage on the organoleptic, nutritional and physicochemical properties of fluids foods[11]. In addition, *Alicyclobacillus acidoterrestris* is a thermoacidophilic, non-pathogenic and spore-forming bacterium which is capable of surviving the pasteurization process[1]. Therefore, this bacteria has been frequently involved in spoilage incidents related to high-acid fruit and vegetable products[34], as it can produce a taint compound, identified as guaiacol, which causes an offensive smelling ''smoky'', ''antiseptic'' or ''disinfectant''-like flavour[18]. Not being associated with secondary gas or acid production, this spoilage is hard to spot with the naked eye; but it might show an increase in turbidity and sediment formation[35].

Emerging preservation processes are being explored and implemented to provide safe, fresher-tasting, nutritive foods without the use of heat or chemical preservatives[22]. In the last decades, a wide range of contemporary physical factors have been intensely investigated with the purpose of inactivating *A. acidoterrestris* spores, encompassing high pressure CO_2[7], microwave[17], high electric field-alternating current[36], ultraviolet light[3] (UV-C), pulsed light (PL)[9,13], among others.

PL has been increasingly used since 1996 when the US Food and Drug Administration (FDA) approved its use to sterilize, sanitize or reduce microbial load in foods, food packaging materials, as well as surfaces, environments, plants, devices and media (water, air) involved in food processes[2]. The implementation of PL for microbial inactivation has gained interest because of the very short treatment time required to achieve a desired microbial decontamination[21]. This technology is based on the application of short intense pulses (100–400 μs) of a broad spectrum between 100 and 1100 nm with 54% of emitted energy in the UV range[19,28]. The germicidal action of PL has been attributed to three different mechanisms which may coexist: the photochemical effect, encompassing the UV fraction, which is responsible for the formation of thymine dimmers that impair cell replication[19]; the photothermal effect, caused by localized overheating of microbial cells during PL treatment[15,40], and the photophysical effect, which induces structural damages in microbial cells due to the pulsing effect[25]. The relative importance of each mechanism would depend on the fluence imparted to the food and target microorganism[19].

PL effectiveness has been attributed mainly to food transparency to the desired light wavelengths. Additionally, other parameters such as turbidity, particle size, suspended solids, presence of particulate materials, dose, composition of the emission spectrum, sample volume, the number and design of lamps have a direct relevance and affect the sample–light interaction[21].

Contamination level of food materials has demonstrated to influence the effectiveness of a wide range of emerging technologies, such as pulsed electric fields[27], ozone[25], high hydrostatic pressure[41], among others. Different responses in inactivation effectiveness were reported by increasing the initial inoculum level, depending on the applied factor and matrix. In particular, the effect of inoculum size in PL effectiveness has not been thoroughly studied. The literature reports controversial effects, either increasing[20] or decreasing PL decontamination[37] while decreasing initial inoculum size.

The aim of the present work was to study the influence of initial contamination level on *A. acidoterrestris* ATCC 49025 spores in apple juice treated by PL. Furthermore, the suitability of Weibull and biphasic models to quantitatively characterize PL inactivation kinetics for a range of different inoculum sizes was analyzed. Additionally, response surface methodology was employed to optimize PL processing time according to the initial inoculum size and the required spore log reductions. To determine the influence of inoculum size (IS) on the decontamination efficiency of PL processing, three different initial spore concentrations (10^3, 10^4 and 10^6 CFU/ml) were inoculated in a 100 mm Petri dish.

Materials and methods

Spore production and inoculum preparation

Experiments were performed using *A. acidoterrestris* ATCC 49025 spores. The initial inoculum was prepared by transferring a loopful of a fresh stock culture maintained in *Bacillus acidoterrestris* medium (BAM) to an Erlenmeyer-flask

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