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Inactivation of *Salmonella* typhimurium and *Lactobacillus plantarum* by UV-C light in flour powder

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ABSTRACT

This research evaluates the potential use of ultraviolet C light (UV-C) as a decontamination method for powdered foods, particularly of refined flour. This technology's lethal effectiveness was evaluated on *Salmonella enterica* subsp. *Enterica* serotype Typhimurium and *Lactobacillus plantarum* in wheat flour, and in laboratory liquid media of different a_w and turbidities to evaluate the action mechanisms of UV-C light in powdered products. Initial results showed a large variability of lethality in flour, obtaining between 0.2 and 3.0 $\log_{10}$ cycles of inactivation. Results obtained in laboratory media and SEM analysis of contaminated flour indicated that the variability was due to a shadow effect on the efficacy of UV-C light and not due to the low water a_w of the flour or starch content. Based on these conclusions, a 2-m vertical tunnel with twelve 480 W UV-C lamps was designed to treat flour by forming a continuous cloud of dust (0.05–2.4 kg/h). Inactivation levels of 4.0 to 1.7 $\log_{10}$ cycles of the population of *L. plantarum* in flour were achieved at flow rates of 0.2 and 2.4 kg/h respectively, with a maximum residence time of 4 s.

Industrial relevance: This investigation demonstrated the lethal efficacy of the application of UV–C light to inactivate microorganisms, both pathogenic and spoilage, present in flour. 4-log10 cycles of inactivation of both *Salmonella* Typhimurium and *Lactobacillus plantarum* were inactivated with UV-C treatments. A UV-C facility was built up which enabled to treat flour in continuous conditions creating a cloud of dust with treatments of 4 s and lethalities of 4-log10 reductions.

1. Introduction

Traditionally, microbial contamination in powders has not been considered an important problem due to their low water activity (a_w) which limits microbial growth during storage (FDA, 2015). Microorganisms are not inactivated at low levels of a_w; instead, however, they become highly resistant to standard decontamination procedures such as heat (Laroche, Fine, & Gervais, 2005). For this reason, microbial contamination is becoming a subject of ever-increasing concern: in particular, contamination caused by pathogenic microorganisms that are present in the raw powder material and which is used in the elaboration of foodstuffs. This problem is remarkable in flours since several studies have found counts from 3 to 5 $\log_{10}$ cycles of total viable counts (Bullerman & Bianchini, 2009; Sauer, 1992; Victor et al., 2013). Other studies define *Salmonella* spp., *Escherichia coli* and *Bacillus cereus*, as pathogenic, and *Lactobacillus* spp., as spoilage, as the microorganisms of major interest in flour and wheat (Berghofer, Hocking, Miskelly, & Jansson, 2003; Eyles, Moss, & Hocking, 1989; Richter, Dorneanu, Eskridge, & Rao, 1993).

Wheat used for flour production is traditionally stored at low humidity prior to use. Immediately before the wheat enters the mill, water is added to increase the wheat's moisture and a_w. This step increases the outer bran layer's plasticity, preventing its fracture during milling and facilitating its separation from the flour in the course of the milling process (Berghofer et al., 2003). This step can nevertheless be a source of contamination. Consecutive milling and sifting processes are performed to obtain refined flour; these processes generate a considerable amount of heat. Moisture condensation can thereby lead to the build-up of flour residues inside the equipment, where microorganisms can accumulate and eventually contaminate the milled products (Berghofer et al., 2003).

As a_w of these products is low, dry heat treatments are ineffective in ensuring microbial decontamination; moist heat treatments cannot be used either, since they might alter the product's characteristics. Thus there is a need to find alternatives to heat for the decontamination of flours and powders. One of these potential alternatives would be UV-C light.

UV-C light has been used in the decontamination of air, surfaces and water. Moreover, the food industry has recently displayed an increasing interest in UV-C light for the hygienization of liquid and solid foodstuffs, since UV-C radiation is capable of inactivating pathogenic and spoilage

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bacteria while only minimally affecting the food's nutritional and sensorial properties. Furthermore, it consumes less energy than other non-thermal food pasteurization technologies (Gayan, Serrano, Pagan, Álvarez, & Condón, 2015; Geveke, 2005; Guerrero-Beltran & Barbosa-Canovas, 2004). Since the key target of UV-C light is DNA, the effect on cell envelopes is minimized. Thus it has been shown that product characteristics such as pH and a_w are not affected by microbial inactivation using UV-C light (Fine & Gervais, 2004; Gayán, Serrano, Raso, Álvarez & Condón, 2012; Gayán, Monfort, Álvarez, & Condón, 2011; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004). This makes UV-C light one of the most promising non-thermal technologies for the decontamination of powdered foods with low a_w, for example flour. However, other factors – for example, process parameters (dose, wavelength), microbial characteristics (intrinsic and extrinsic factors), and other food characteristics such as the absorption coefficient and turbidity – could affect the efficiency of UV-C light (Gayán, Condón, & Álvarez, 2014). All these factors can limit UV-C light lethal effectiveness on microbes.

Hence, the main objective of this study was to assess the potential of UV-C light for flour decontamination, and to determine how a series of factors might affect the treatment's lethal effectiveness. To carry out the investigation, *Salmonella* Typhimurium was used as a reference pathogenic microorganism, since *Salmonella* spp. has been the main pathogenic bacterium isolated in products of this kind. To evaluate our constructed prototype's lethal effectiveness in continuous conditions *Lactobacillus plantarum* was used since it is a non-pathogenic but spoilage microorganism of interest in these products (Bullerman & Bianchini, 2009). Treatments were applied using different methods, in different setups, and in both solid and liquid media, in order to identify which properties and characteristics of the product had more influence on UV-C light's lethal effectiveness against microorganisms.

2. Material and methods

2.1. Bacterial culture and media

The strains of *Salmonella* Typhimurium STCC 878, *and Lactobacillus plantarum* STCC 748 used in this investigation were provided by the Spanish Type Culture Collection (STCC). Bacterial cultures were maintained at −80 °C in Tryptone soy broth (TSB; Oxoid, Basingstoke, Hampshire, England) for *Salmonella* Typhimurium, and MRS broth (Oxoid) for *L. plantarum*, with 25% added glycerol. A broth subculture was prepared by inoculating 5 mL of TSB supplemented with 0.6% (*w*/*v*) yeast extract (Oxoid) – TSBYE - or MRS broth (Oxoid) respectively, with a single colony obtained from solid growth media. *Salmonella* Typhimurium subcultures were incubated at 35 °C overnight. From the subculture, a 250 mL Erlenmeyer flask containing 50 mL of TSBYE was inoculated into a concentration of 10^6 CFU/mL. Then the culture flask was incubated at 35 °C in a shaking incubator at 150 rpm (Heidolph Instruments, Vibramax 100, Schwabach, Germany) for 24 h until stationary growth phase ($1x10^9$ CFU/mL). On the other hand, *L. plantarum* subcultures were incubated at 37 °C in an anaerobic chamber (MACS VA500, microaerophilic workstation, Don Whitley Scientific, West Yorkshire, UK) for 12 h. Then a 250 mL Erlenmeyer flask containing 50 mL of MRS broth was inoculated into a concentration of 10^6 CFU/mL. The culture flask was subsequently incubated in an anaerobic chamber with 150 rpm agitation at 37 °C during 48 h until stationary growth phase ($1x10^9$ CFU/mL).

2.2. Treatment media and analytical measurements

Refined wheat flour was bought in a local supermarket (Comercial Gallo, S.L., Spain). The water activity and humidity of the flour was 0.56 and 12% (at 21.9 °C), respectively. McIlvaine citrate–phosphate buffers (Dawson, Elliot, Elliot, & Jones, 1974) of pH 7 with different degrees of water activity (>0.99, 0.90, 0.80, 0.66, and 0.40), of absorption coefficients (4, 8, 12, 15, and 40 cm^{-1}), and of turbidity (0, 128, 294, 627, and 1130 NTU), along with a saturated solution of soluble starch of $a_w > 0.99$ (Sigma-Aldrich Company Ltd. The Old Brickyard, New road, Gillingham, United Kingdom), were used as laboratory treatment media. Buffers of different a_w, absorption coefficients and turbidities were obtained by adding different quantities of glycerol (Panreac, Barcelona, Spain), tartrazine (Sigma-Aldrich, St. Louis MO, USA), and different proportions of flour respectively.

The treatment media's absorption coefficient was determined by following the procedure described in Koutchma, Forney, and Moraru (2009). Turbidity was measured using an HI 83749 nephelometer (Hanna Instrument, Szeged, Hungary). The pH was adjusted by using a pH meter BASIC 20 (Crison Instrument, Barcelona, Spain). Water activity was measured at room temperature with a dew point instrument (Water Activity System mod. CX-1, Decagon Devices, Pullman, WA, USA).

2.3. Sample preparation

Although initially a series of different flour contamination methods were evaluated to optimize and standardize the procedure, in this section it is described the methodology selected for using in this investigation. 100 g of refined flour were distributed homogeneously on a 50 cm^2 stainless steel tray. Then 10 mL of pure culture with a concentration of 10^9 CFU/mL were added using a bottle sprayer. The contaminated product was subsequently dried at room temperature for 24 h in a sterile laminar flow chamber (Telestar mini-V/PCR, Telestar Technologies, S.L., Terrasa, Spain) in order to remove excess moisture. a_w before and after contamination and drying varied ±0.01.

2.4. UV-C treatments

UV-C treatments were carried out in different setups according to treatment media and research goal. The effects of a_w (solutions of $a_w > 0.99$, 0.90, and 0.80) and starch on the UV-C microbial lethality were evaluated in the equipment described in Gayán et al. (2011) which in this investigation has been renamed as UV-1 system. In short, this facility consisted of eight annular thin-film continuous flow-through reactors connected in series and equipped with a feed tank and a peristaltic pump (ISM 10785, Ismatec, Glattbrugg, Switzerland). Each reactor consisted in a low-pressure mercury vapor lamp (8 W; model TUV 8WT5, Philips, USA), enclosed in a quartz tube (20 mm of outer diameter) with an annular treatment gap of 2.5 mm, thereby allowing the continuous flow of treatment liquids at 8.5 L/h.

To evaluate the effect of absorbance coefficient along with turbidity on the UV-C lethality, a more simple installation, here named the UV-2-system, was used. In the UV-2 system, 50 μL of bacterial culture in stationary phase were added to a Petri dish containing 15 mL of treatment media (McIlvaine buffer of pH 7). The Petri dish was located at a distance of 5 cm from two 32 W UV-C lamps (VL-208G, Vilber, Germany) with a fluence of 8.0 ± 0.2 mW/cm^2 measured with a UVX radiometer (UVP, LLC, Upland, CA). The contaminated treatment media was agitated with a magnetic stirrer at 250 rpm, and 250 μL samples were taken at preset treatment times. The entire system was placed inside an incubator with an air temperature of 24 °C. The same methodology and equipment were also used to treat and measure the McIlvaine solutions of a_w of 0.66 and 0.40 respectively, since they could not be pumped through the UV-1 system due to their viscosity. A reference solution of $a_w > 0.99$ was also treated in the UV-2-system for comparison purposes.

In order to perform the treatments on flour, two different setups were used: one to apply treatments in batch and a second one in continuous flow. Initial experiments were carried out in a batch facility: the UV-3-system. It consisted of a vibrating plate of 15 cm diameter attached to a Vortex Genius 3 test tube shaker (Carl Roth GmbH + Co.