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Characterization and effectiveness of short-wave ultraviolet irradiation reactors operating in continuous recirculation mode to inactivate *Saccharomyces cerevisiae* in grape juice

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ABSTRACT

Short-wave ultraviolet (UVC) irradiation is an emerging process that has been reported as an effective method for inactivating bacteria that contaminate water. Research regarding this technology in foods has focused on treating certain liquids inoculated with particular target microorganisms. We evaluated different UVC reactor arrangements with recirculation to inactivate *Saccharomyces cerevisiae* in grape juice. Tested juice was inoculated with *S. cerevisiae* and processed at three flow rates (5.2, 17.1, or 31 mL/s) during 60 min. Selected arrangements of UVC reactors were assessed by varying the length (30 or 80 cm) of the UVC lamp (254 nm), the number of lamps (1 or 2), and the thickness of the gap in the reactor (1.0 or 0.5 cm) for the 30 cm UVC lamp. Additionally, UVC dosages of studied arrangements were evaluated through dye degradation in order to determine the effect of flow rate in the recirculating systems. In UVC reactors consisting of a single lamp, approximately 2.5 log-cycle reductions were achieved with flow rates of 17.1 or 31 mL/s, whereas at 5.2 mL/s only 2 log-cycle reductions were attained. With two UVC lamps in series, 5 log-cycle reductions were achieved after 45 min of treatment regardless the length of the lamp but only at the high flow rates tested. With the reactor of the smallest tested thickness, 6 log-cycle reductions were achieved at 17.1 mL/s or 31 mL/s. In general, reduction of juice film thickness allowed greater reductions of *S. cerevisiae*; furthermore, important differences were observed among the evaluated systems when comparing the effect of flow rate.

1. Introduction

Nowadays, consumers are demanding processed food products with similar characteristics to their fresh counterparts. Thus, emerging technologies such as high hydrostatic pressures, pulsed electric fields, low frequency ultrasound, and ultraviolet light irradiation have been investigated in order to obtain this type of products. Short-wave ultraviolet (UVC) irradiation has been reported as effective for inactivating bacteria that contaminate water and surfaces of various materials, with the advantage that does not generate chemical residues (Quek and Hu, 2008). The antimicrobial effect of UVC on microorganisms occurs at the DNA level causing breaking of DNA bonds; therefore, microbial cell viability is compromised. The photoproducts (pyrimidine nucleotide bases) generated by the application of UVC, block DNA transcription and replication; even more, inhibit cell functions and thus, cause cell death (Guerrero-Beltrán and Barbosa-Cánovas, 2004).

Even though UVC is commonly utilized for air and water treatment as well as for surface decontamination, its use for treating liquid foods is still limited (Koutchma, 2009). In 2000 the US Food and Drug Administration (FDA) approved the use of UVC as an alternative treatment to thermal pasteurization of fresh and cloudy juices; this led to a rising appeal on this technology; however, it was also advised that to ensure the effectiveness of the process at least 5 log cycle reductions of the microorganism of interest must be obtained for every particular juice (FDA., 2001).

There are different types of liquid food products that have been treated with UVC such as pineapple (López-Díaz et al., 2013), apple (Caminiti et al., 2012), and grape (Guerrero-Beltrán et al., 2009) juices, among others. These liquid foods usually insufficiently transmit UV light; thus, affecting microbial inactivation for different reasons such as the quantity of suspended soluble and non-soluble solids present in the liquid. It is well-known that UVC has a limited penetration depth, so it is necessary to consider different factors when designing UVC reactors

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such as the thickness of the layer of irradiated liquid, type of flow, and mass flow, among others (Bintsis et al., 2000). The geometric configuration of the UVC irradiation device is another critical factor to ensure proper microbial inactivation (Gayán et al., 2014). For these reasons, some researchers have tested different geometric configurations of the UVC-reactors to produce a thin layer of liquid along the UVC lamp and/or generate turbulent flows (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Recently, Abdul Karim Shah et al. (2016) reviewed fruit juice processing by means of UV technologies, describing microbial inactivation, food quality and sensory effects, as well as the combination of UVC with other thermal and non-thermal methods (high pressures, pulse electric fields, pulsed light, sonication, etc.) to maximize process effectiveness. A comprehensive revision of the critical factors that affect UVC light microbial inactivation and the advances on design of UVC reactors for liquid processing is also available in Koutchma (2009). Both reports highlight that although the usage of UVC has been documented by several authors during the last decade, it is not straightforward to generalize the effects of UVC treatments since their effectiveness rely on many different factors, including food characteristics (optical and physical properties), type of microorganisms, process conditions (temperature, flow rate, etc.), and operation mode (continuous or batch). Therefore to produce safe foods with similar characteristics to their fresh counterparts, it is necessary to further study diverse types of arrangements of UVC reactors in order to properly design them for future escalation. Thus, the objective of this study was to evaluate the effect of selected UVC reactor arrangements with recirculation in the response of *Saccharomyces cerevisiae* inoculated in grape juice to suggest suitable reactor arrangements that ensure at least 5 log cycle reductions.

2. Materials and methods

2.1. Inoculum preparation and juice characteristics

S. cerevisiae was grown in 100 mL of sterile Sabouraud broth (BD Difco™, Sparks, MD) at 35 °C during 48 h for each test and then 1 L of commercial pasteurized grape juice was inoculated with enough inoculum to obtain ≈ 10^6 CFU/mL. For juice characterization, its color was determined with a colorimeter (CR-400 Konica Minolta, Netherlands), where the parameters *L*, *a*, and *b* on the Hunter scale were obtained. Its pH was measured with a potentiometer (PH10, Conductronic, Mexico) and the total soluble solids (°Brix) were determined with a digital refractometer (PR-101, ATAGO®, Japan). Studied juice pH and °Brix values were 3.46 ± 0.05 and 13.10 ± 0.10, respectively. Juice density (ρ) of 1057 kg/m^3 was obtained by means of a glass picnometer (AOAC, 2012) while a viscosity value (μ) of 0.00228 kg/m·s at 20 °C was determined using a Cannon-Fenske viscometer. Every measurement was performed by triplicate.

2.2. UVC treatment

UVC equipment. The AquaPlus™ commercial UVC processing unit (Models 4SS & 12SS, Industrias Mass, Mexico) made of stainless steel was utilized in this study. The effects of the length (30 or 80 cm) of the UVC lamp, the number (1 or 2) of lamps, and the effect of the thickness of the gap in the reactor (1.0 or 0.5 cm) around tested lamps were evaluated. The stainless steel case diameter (Ds) was 4.8 cm for the arrangements with 1 cm of annular space while for the arrangement with 0.5 cm of annular space the Ds was 3.5 cm. A total of five different arrangements were studied, which are portrayed in Fig. 1.

The UVC lamps from LightSources Inc, USA (30 cm, Model GPH357T5L and 80 cm, Model GPH793T5L) of 17 and 38 W, according to the manufacturer, were warmed up for 15 min before circulating the studied juice. The intensity or UVC fluence rate was measured at the center and on the surface of quartz cylinders that covered the lamps using a digital radiometer (Cole Palmer Instrument Co., IL, USA), being 10.3 ± 0.15 mW/cm^2 and 11.1 ± 0.10 mW/cm^2 for lamps of 30 and 80 cm, respectively. The diameter of the quartz cylinder (Dq) was 2.5 cm and this was the same for every one of the tested arrangements.

Juice processing. Pasteurized grape juice was purchased at a local supermarket in Puebla, Mexico and inoculated as stated before every time it was processed. The juice flowed into the annulus between the equipment stainless steel tube and the UVC lamp quartz cylinder and it was recirculated using a peristaltic pump (MasterFlex 7553-71, Cole-Parmer, Vernon Hills, IL) at three different flow rates (5.2, 17.1, or 31 mL/s) during 60 min operating in a continuous recirculation mode. The juice was placed in a double-wall cylindrical vessel and connected to a water-bath (Polystat 1268-24, Cole-Parmer, Vernon Hills, IL) that kept the temperature of the juice at 15 ± 1 °C (Fig. 2). Before each experiment, the system was rinsed with 500 mL of sterile water.

2.3. Microbial analysis and modeling

Samples of 100 μL of the juice were taken at 0, 5, 10, 15, 20, 25, 30, 40, 50, and 60 min of treatment. These samples were plated in potato dextrose agar with an Autoplate spiral plating system (Autoplate 4000, Spiral Biotech, Norwood, MA). Then Petri dishes were incubated at 35 °C during 48 h; afterwards, colonies were counted (Antonio-Gutiérrez et al., 2017) with an automatic colony counter (QCount Model 530, Spiral Biotech). The Weibull model was utilized in this study to describe studied yeast inactivation kinetics; tested model is represented by the following equation:

$$\log \frac{N}{N_0} = -bt^n \tag{1}$$

Where N is the microbial load at time t and N_0 is the initial microbial load while b and n are parameters of the model (Peleg and Cole, 1998). Weibull parameters were determined using non-linear regression (Flores-Cervantes et al., 2013).

2.4. UVC fluence estimation and flow regime

Different methods were evaluated for determination of the UVC fluence delivered by the studied arrangements. UVC fluence estimation can be computed per area and per pass (Henrique et al., 2016) or per treated milliliter (Keyser et al., 2008).

The UVC fluence per area and per pass (D_A) was calculated according to Henrique et al. (2016) with the following equation:

$$D_A = I^*\tau \tag{2}$$

Where I is the intensity of the lamps or UVC fluence rate (mW/cm^2) and τ the theoretical residence time. The theoretical residence time was calculated by dividing the volume of the studied arrangement (annular space) by the flow rate.

Then, the total UVC fluence per area (TD_A) was calculated with the next equation:

$$TD_A = D_A * Rc \tag{3}$$

Where Rc is the total number of times that the juice re-circulates in the system. This parameter was calculated by dividing the total treatment time (3600 s) by τ.

Whereas the UVC fluence per milliliter and per pass (D_L) was determined according to the method proposed by Keyser et al. (2008) with the following equation:

$$D_L = \text{Total UVC output per lamp (W) / Flow rate (mL/s)} \tag{4}$$

Then the total UVC fluence per milliliter (TD_L) was also calculated:

$$TD_L = D_L * Rc \tag{5}$$

The total UVC lamp output was calculated by multiplying the intensity or fluence rate of the lamps by the outer surface area (A) of the quartz cylinder. The surface area for studied 30 cm lamps was 235.6 cm^2 while