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Modeling optimal process conditions for UV-heat inactivation of foodborne pathogens in liquid foods

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ABSTRACT

The combination of ultraviolet radiation and heat (UV-H treatment) has been demonstrated as a promising strategy to overcome the limited UV germicidal effect in fruit juices. Nonetheless, there are so far no data regarding the efficacy of the combined process for the inactivation of bacterial foodborne pathogens in other liquid foods with different pH and composition. In this investigation, the optimum UV-H processing conditions for the inactivation of *Escherichia coli*, *Salmonella* Typhimurium, *Listeria monocytogenes*, and *S. aureus* in chicken and vegetable broth, in addition to juices, were determined. From these data models that accurately predict the most advantageous UV-H treatment temperature and the expected synergistic lethal effect from UV and heat resistance data separately were constructed. Equations demonstrated that the optimum UV-H treatment temperature mostly depended on heat resistance, whereas the maximum synergistic lethal effect also was affected by the UV resistance of the microorganism of concern in a particular food.

1. Introduction

Most processed foods are thermally treated to inactivate pathogenic and spoilage microorganisms as well as enzymes to obtain safe products with prolonged shelf life. However, heat treatment may lead to undesirable changes in physicochemical, nutritional, and sensorial properties of foods. To satisfy current consumers' demand for high-quality foods which are microbiologically safe, the food industry has been challenged to develop more appropriate methods for food hygienization. Among the non-thermal technologies developed in the last few decades, ultraviolet (UV) based treatments are one of the most encouraging alternatives to the thermal pasteurization of liquid foods. UV radiation is lethal for most type of microorganisms, minimizes the loss of nutritional and sensorial quality (Caminiti et al., 2012; Tran and Farid, 2004), does not generate chemical residues (Guerrero-Beltrán and Barbosa-Cánovas, 2004), and it requires low energy consumption compared to other non-thermal pasteurization processes (Geveke, 2005). In fact, the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) of the USDA revised the definition of "pasteurization", and included UV radiation as an alternative to heat for pasteurization purposes (NACMCF, 2006).

Short-wave UV or UV-C radiation (200–280 nm) is the most germicidal UV region, and its maximum lethal effect occurs at wavelengths between 250 and 270 nm (Kowalski, 2009). Hence, low-pressure mercury vapor lamps are the most frequently used UV sources for disinfection purposes, since they emit quasi-monochromatic UV-C radiation at 253.7 nm. The UV-C lethal effect is attributed to the formation of DNA photoproducts, especially cyclobutane pyrimidine dimers and 6,4-photoproducts, which distort DNA molecules inhibiting transcription and replication, and eventually leading to cell death (Sinha and Häder, 2002).

Despite its advantages, the application of UV technology in the food industry is still limited because radiation only penetrates a very short depth inside liquid food surfaces. The UV transmittance of a treatment medium depends on the absorptivity of the liquid and the present amount of suspended solids. Color compounds, soluble solids, and suspended particles absorb UV photons reducing UV light's penetration depth into liquid surfaces

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(Koutchma et al., 2004). In addition, suspended solids cause reflection and scattering phenomena that also contribute to decrease the penetration depth (Koutchma et al., 2004). Consequently, extremely high UV doses are needed to achieve the U.S. Food and Drug Administration (2001) requirement of 5 Log_{10} reductions in low UV transmittance foods, which decrease the sensorial acceptability and nutritional value (vitamin C content and antioxidant capacity) of the fresh product (Caminiti et al., 2012; Tran and Farid, 2004).

To overcome this limitation, turbulent flow reactors have been designed to optimize the effect of UV radiation (Franz et al., 2009; Koutchma et al., 2007). Another alternative is to develop "hurdle" approaches combining UV radiation with other non-thermal technologies or conventional preservation methods at moderate intensity (Char et al., 2010; Walkling-Ribeiro et al., 2008). One of the most promising hurdle strategies is the combination of UV-C radiation with mild heat (UV-H treatment), since UV inactivation increases at temperatures between 45 °C and 60 °C (Gayán et al., 2011; Geveke, 2008). Recently, it has been demonstrated that the inactivation of *Escherichia coli* by UV-H treatment in apple and orange juice resulted from a synergistic effect, and that the magnitude of such effect depended on the treatment temperature (Gayán et al., 2013a, 2012). However, the occurrence of this synergistic lethal effect in other liquid foods with different pH, chemical composition, and absorptivity has not been further investigated. Moreover, this knowledge remains to be extended to the UV-H inactivation of other foodborne pathogens. Therefore, it is not possible to establish any relationship between the temperature of maximum synergistic lethal effect and the magnitude of this synergy with the UV and heat resistance of the microorganism of concern in different food products.

Today it is not possible to predict whether UV-H processing is advantageous for the pasteurization of a given liquid food or which the optimum UV-H treatment temperature is. This research was initiated to address these gaps in knowledge. For this purpose, we completed data previously published on the effect of treatment temperature on the UV-C inactivation of *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus* suspended in orange and apple juice, and vegetable and chicken broth. Subsequently, the treatment temperature most beneficial for the UV-H inactivation of each microorganism in each product was optimized. Finally, mathematical models that predict the optimum UV-H treatment temperature and the expectable synergistic effect from UV and heat resistance data were developed.

2. Materials and methods

2.1. Bacterial culture and media

The strains *E. coli* STCC 4201, *Salmonella enterica* subsp. *Enterica* serovar Typhimurium STCC 878, *Listeria monocytogenes* STCC 5672, and *S. aureus* STCC 4465 were obtained from the Spanish Type Culture Collection (STCC). The bacterial cultures were maintained frozen at −80 °C in cryovials. A broth subculture was prepared by inoculating 10 mL of tryptone soy broth (Biolife, Milan, Italy) supplemented with 0.6% (w/v) yeast extract (Biolife) (TSBYE) with a colony grown on tryptone soy agar (Biolife) supplemented with 0.6% (w/v) yeast extract (TSAYE). The subculture was incubated at 35 °C for 6 h in a shaking incubator. With this subculture, a 250 mL Erlenmeyer flask containing 50 mL of TSBYE was inoculated to a concentration of 10^4 CFU/mL and incubated under the same conditions for 24 h, which resulted in stationary-phase cultures containing approximately 10^9 CFU/mL.

2.2. Treatment media and analytical measurements

Commercially sterile apple juice (García Carrión S.A., Spain; absorption coefficient = 24.0 ± 2.1 cm^{-1}, turbidity = 7.7 ± 4.9 NTU, pH = 3.35 ± 0.09), orange juice (Dafsa, Spain; absorption coefficient = 81.7 ± 8.4 cm^{-1}, turbidity = 4195 ± 487 NTU, pH = 3.56 ± 0.04), vegetable broth (Interal S.A., Spain; absorption coefficient = 27.6 ± 4.4 cm^{-1}, turbidity = 2315 ± 354 NTU, pH = 5.75 ± 0.38), and chicken broth (Interal S.A., Spain; absorption coefficient = 22.2 ± 3.8 cm^{-1}, turbidity = 3425 ± 898 NTU, pH = 5.39 ± 0.32) were used as treatment media. The sterility of each product was tested plating an aliquot of 1 mL in the recovery medium before use. Absorbance of media was measured at 254 nm using a Unicam UV500 spectrophotometer (Unicam Limited, Cambridge, UK). Liquid foods were diluted and absorptivity was evaluated using quartz cuvettes (Hellma, Müllheim, Germany) with path lengths of 1, 2, and 10 mm. The absorption coefficient of samples was determined from the slope of the absorbance versus the path length and corrected by the dilution factor. Turbidity was measured using a HI 83749 nephelometer (Hanna Instrument, Szeged, Hungary) and expressed in Nephelometric Turbidity Units (NTU). The pH was measured with a pH meter BASIC 20 (Crison Instrument, Barcelona, Spain).

2.3. UV-H treatments

UV treatments were carried out in the equipment previously described by Gayán et al. (2011). The system consisted of eight individual annular thin-film flow-through reactors connected sequentially and fed by a peristaltic pump (ISM 10785, Ismatec, Glattbrugg, Switzerland). Each reactor consisted of a low-pressure mercury vapor lamp (8 W input power; TUV 8WT5, Philips, USA), that emitted 85% of the energy at a wavelength of 254 nm, fixed at the axis of an outer glass tube (25 mm of inner diameter) and enclosed by a quartz tube (20 mm of outer diameter) to prevent the lamp's direct contact with the treatment medium. In the annular gap (2.5 mm), a stainless steel coil spring was installed in order to improve the flow's turbulence. Outside and inside coil diameters of the spring were 23 and 25 mm, respectively, and its length and pitch were 270 and 10 mm, respectively.

To conduct UV-H treatments, the entire unit was submerged in a 90 L water bath heated by the circulating water of a peripheral thermostatic bath (Kattebad K12, Huber, Offenburg, Germany). A heating/cooling coil exchanger was placed before the inlet of the first reactor. Two thermocouples (639 K, Crison Thermometer, Barcelona, Spain) that were fitted to the inlet of the first reactor and the outlet of the last reactor allowed for the control of temperature.

The treatment medium was inoculated with the bacterial suspension to achieve approximately 10^7 CFU/mL. Higher cell concentrations decreased the effectiveness of UV treatment probably because of increased turbidity and absorption coefficient of the medium (data not shown). Contaminated medium was pumped through the equipment at a flow rate of 8.5 L/h. At this flow rate, the ratio between the mean residence time obtained from the residence time distribution curve ($\bar{t}$) and the theoretical residence time calculated by the flow rate and the volume reactor ($\hat{t} = V/Q$) was close to the unit (0.944), indicating that the flow approached to a turbulent regime (Gayán et al., 2011). When the flow rate stabilized, samples were withdrawn through the sampling valves placed at the outlet of each reactor, and they were immediately diluted and pour-plated. The UV dose actually delivered to the treatment medium was estimated by the chemical actinometer iodide-iodate following the indications of Rahn et al. (2003). The actinometer buffer was pumped through the installation at the treatment flow rate and the increase in absorbance (352 nm) was determined at the outlet of