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# Study of the inactivation of some microorganisms in turbid carrot-orange juice blend processed by ultraviolet light assisted by mild heat treatment

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## ABSTRACT

The aim of this study was to evaluate the effect of short wave ultraviolet light (UV-C, 0–10.6 kJ/m<sup>2</sup>) assisted by mild heat treatment (UV-C/H; 40, 45 or 50 °C) on the inactivation of *Escherichia coli*, *Saccharomyces cerevisiae* and *Pseudomonas fluorescens* in freshly squeezed carrot-orange juice blend. In addition, the suitability of three conceptually different models was analyzed to characterize the inactivation kinetics. All treatments provoked moderate to high microbial inactivation depending on temperature and microorganism (2.6–6.0 log reductions). The use of UV-C assisted by mild heat treatment notably improved inactivation compared to single UV-C. Synergistic inactivation effects on *E. coli* and *P. fluorescens* were observed at combined UV-C/H (45 and 50 °C). Gompertz and Geeraerd models allowed a better fit and more accurate parameter estimation compared to the Weibull model.

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## 1. Introduction

Consumer demand towards fresh-like, ready-to drink and healthier fruit juices has increased in the last decades mainly due to the presence of antioxidants, vitamins and minerals. These compounds play an important role in the prevention of heart diseases, cancer and diabetes (Matthews, 2006). In particular, carrot juice is rich in most of the natural antioxidants, including carotenoids, phenolics, vitamin C and tocopherol (Sharma et al., 2012). It is the main natural source of  $\beta$ -carotene, which protects against the free radicals generated endogenously through normal diet and metabolic activity, as well as from environmental sources.  $\beta$ -carotene provides this protection by acting as a strong quencher of singlet molecular oxygen and peroxy radical scavengers (Schafer et al., 2002). Orange juice is a popular product representing a substantial source of vitamin C (Polydera et al., 2003). The antioxidant effect of vitamin C has been the focus of many research studies. It has

been concluded that vitamin C helps in the prevention of cancer (Byers and Perry, 1992; Wittes, 1985).

Although heat pasteurization is the most commonly used technique for fruit processing, as it ensures safety and long product shelf life, it is well-known that traditional thermal processes cause significant damage on organoleptic, nutritional and physicochemical properties of fluid foods (Elmnasser et al., 2008). In order to prevent those undesirable effects, a wide range of emerging technologies has been investigated in the last decades for alternative processing of fruit and vegetable juices. The list encompasses technologies with different levels of development, going from those already implemented in industry such as high hydrostatic pressures (Dede et al., 2007; Buzrul et al., 2008), those approved by the FDA and under implementation, like ultraviolet light (Koutchma et al., 2007; Unluturk et al., 2010) ozone (Patil et al., 2009; García Loredó et al., 2015), and pulsed light (Pataro et al., 2011; Ferrario et al., 2013, 2015); to those that are less developed such as ultrasound (Char et al., 2010a; Ferrante et al., 2007).

Short-wave ultraviolet light (UV-C) is one of the most promising low-cost and energy efficient non-thermal technologies, used for decontamination of freshly squeezed juices. It encompasses the UV spectrum range from 200 to 280 nm, being lethal to a large variety of microorganisms, without generating chemical residues (Baysal

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et al., 2013). In particular, exposure to UV light results in the cross-linking of neighboring pyrimidine nucleotide bases in the same deoxyribonucleic acid (DNA) strand, eventually causing cell death (Gabriel, 2012). UV-C pasteurization is affected by many different factors such as UV light source choice, reactor design, flow rate, type of liquid, viscosity, density, UV-C light absorptivity, presence of soluble and insoluble solids, and particle size (Koutchma, 2009). Until recently, the implementation of UV-C as a decontamination technique has been limited to clear liquid foods and beverages. It has been successfully applied, achieving more than the 5 log reductions required by the US Food and Drug Administration (FDA, 2000), for the inactivation of *E. coli* in clear apple juice encompassing a broad range of UV-C doses from 18.7 to 531 kJ/m<sup>2</sup> (Keyser et al., 2008; Franz et al., 2009; Char et al., 2010a; Caminiti et al., 2012). Moreover, more than 5 log reductions of *Salmonella* Typhimurium were also achieved in pineapple juice applying an UV-C dose of 137.5 kJ/m<sup>2</sup> (Mansor et al., 2014), and *Alicyclobacillus acidoterrestris* spores in white grape juice (1.31 mW/cm<sup>2</sup>) (Baysal et al., 2013), among others. UV-C possesses notoriously less effectiveness in highly turbid juices due to the presence of large amounts of UV-absorbing compounds and suspended particles. For instance, Char et al. (2010a) obtained only 1.5 log reductions of *Escherichia coli* in UV-C treated freshly squeezed orange juice (18.7 kJ/m<sup>2</sup>). Authors explained this remarkable low efficiency of UV-C disinfection due to the presence of colored compounds and pulp particles which caused poor UV-C light transmission. Baysal et al. (2013) observed only up to 2 log reductions of *A. acidoterrestris* spores in UV-C treated apple juice (1.31 mW/cm<sup>2</sup>), which possessed higher turbidity and absorption coefficient than the white grape juice used in the same study. These particles reduce UV light transmittance, thereby impairing the disinfection process (Gayán et al., 2013). Large suspended particles may also block the incidence of light on the microbial load (Guerrero-Beltrán and Barbosa-Cánovas, 2004). To overcome this limitation, combined processes have been recently designed by applying UV light with the assistance of other processing techniques to achieve maximal benefits in microbial reduction and retention of juice quality (Shah et al., 2016). The combination could be between UV-C technology and heat, other nonthermal technologies, or the addition of chemicals and preservatives. For example, higher inactivation compared to individual treatments, was achieved when applying UV-C light (0.011 kJ/m<sup>2</sup>) combined with ultrasound (20 kHz, 95 μm-wave amplitude) for *S. cerevisiae* in apple juice (López-Malo et al., 2005), and *E. coli* in orange juice (Char et al., 2010a). In addition, UV-C (25 kJ/m<sup>2</sup>) combined with 50 ppm of citral and 1500 of vanillin in orange juice delayed *Zygosaccharomyces bailii*, *E. coli* and *Listeria innocua* recovery during 13 days of refrigerated storage (Ferrario et al., 2011). Moreover, the combination of UV-C (203 kJ/m<sup>2</sup>) and addition of sodium benzoate (250, 500, 1000 and 2000 ppm) for peach nectar processing demonstrated a synergistic inactivation effect on *Aspergillus niger* and *Aspergillus flavus* (Flores-Cervantes et al., 2013). Tan (2012) observed a 5 log reduction of *Listeria innocua* in green guava juice treated by UV-C (0.035 kJ/m<sup>2</sup>) followed by mild heat treatment (55 °C, 60 s). In this context, the use of ultraviolet light assisted by mild heat treatment to reach the desired inactivation effect, represents an alternative for the development of minimally processed turbid juices.

Microbial inactivation by UV-C in liquid media has been extensively characterized in literature. Few authors have reported linear behavior, such as Ochoa-Velasco and Beltrán (2013). However, most authors have reported non-linear survival curves after UV-C processing. Some of them have reported the presence of tail (Baysal et al., 2013; Unluturk and Atilgan, 2014), shoulder (Gayán et al., 2012a) or both (Quintero-Ramos et al., 2004). Several authors have successfully applied the Weibull model, which is based

on the hypothesis that there is a cumulative form of a temporal distribution of lethal events. Therefore, each microbial cell dies or is inactivated at a specific time. Weibull model has shown good applicability for the characterization of microbial inactivation in orange juice or peach nectar (Taze et al., 2015; Flores-Cervantes et al., 2013) and solid matrices like pear slices (Schenk et al., 2008). Other authors have used predictive models which take into account shoulder and/or tail, such as the modified version of Gompertz and Geeraerd models. For general predictive purposes, the Gompertz and Geeraerd models have an important practical advantage over most other models. All the parameters derived from these models have a clear biological and/or graphical meaning, and the three phases of the inactivation curve (shoulder, log-linear phase and tailing region) are easily recognizable. Geeraerd model successfully characterized survival data of pathogenic microorganisms in solid and liquid food matrices subjected to different treatments, such as mild thermal treatment (Geeraerd et al., 2000) and UV-C assisted or not by mild heat (Gayán et al., 2013). Whereas, Gompertz model adequately characterized inactivation curves during a mild thermal treatment combined with vanillin plus citral (Char et al., 2010b), and during ultrasound treatment combined with low weight chitosan addition (Guerrero et al., 2005), among others. Nevertheless, suitability and comparison of these models has not been deeply analyzed in turbid juices treated by UV-C.

The aim of this research was to investigate the effect of UV-C treatment assisted by mild heat treatment (UV-C/H) on the response of some microorganisms of concern inoculated in a carrot-orange juice blend. The suitability of Weibull, Gompertz and Geeraerd models was analyzed to characterize single and combined UV-C processing inactivation kinetics for a range of temperatures and microorganisms.

## 2. Materials and methods

### 2.1. Strains and preparation of inocula

Experiments were performed using *Escherichia coli* ATCC 35218, *Saccharomyces cerevisiae* KE 162 and *Pseudomonas fluorescens* ATCC 49838. Initial *E. coli* inoculum was prepared by transferring a loopful of Trypticase Soy Agar plus 0.6% w/v Yeast Extract (TSAYE, Biokar Diagnostics, Beauvais, France) slant stock culture to a 20 mL of Trypticase Soy Broth supplemented with 0.6% w/v Yeast Extract (TSBYE, Biokar Diagnostics, Beauvais, France). It was incubated at 37 °C under agitation for 18 h until it reached stationary phase. A similar procedure was repeated for *S. cerevisiae* and *P. fluorescens*, for which the initial inocula were prepared by transferring a loopful of fresh stock cultures maintained in Sabouraud Dextrose Agar (SAB, Biokar Diagnostics, Beauvais, France) or Nutrient agar (NA, Biokar Diagnostics, Beauvais, France) to 20 mL of Sabouraud Dextrose Broth (SAB Broth, Biokar Diagnostics, Beauvais, France) or Nutrient broth (NB, Biokar Diagnostics, Beauvais, France), respectively. Incubation was performed at 27 °C for 24 h. All inocula were harvested by centrifugation (1475 g, 5 min) (Labnet, Edison, New Jersey, USA), washed twice with peptone water to obtain a cell density of 10<sup>7</sup>–10<sup>9</sup> CFU mL<sup>-1</sup>. For the inoculation, 5 mL of the microbial suspension was added to 745 mL of carrot-orange juice prior to UV-C treatment.

### 2.2. Carrot-orange juice blend preparation

Fresh carrot juice was manually obtained under aseptic conditions by pressing carrots (*Daucus carota*, var. Chantenay). A household juicer (Ju655, Moulinex, Taipéi, Taiwan, China) was sanitized with 70% (v/v) ethanol and exposed to UV-C for 10 min. Similarly, fresh orange juice (*Citrus sinensis*, var. Valencia) was

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