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# Effects of ultraviolet light emitting diodes (LEDs) on microbial and enzyme inactivation of apple juice

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ABSTRACT

In this study, the effects of Ultraviolet light-emitting diodes (UV-LEDs) on the inactivation of *E. coli* K12 (ATCC 25253), an indicator organism of *E. coli* O157:H7, and polyphneoloxidase (PPO) in cloudy apple juice (CAJ) were investigated. The clear (AJ) and cloudy apple juice were exposed to UV rays for 40 min by using a UV device composed of four UV-LEDs with peak emissions at 254 and 280 nm and coupled emissions as follows: 254/365, 254/405, 280/365, 280/405 and 254/280/365/405 nm. UV-LEDs at 254 nm achieved 1.6 ± 0.1 $\log_{10}$ CFU/mL inactivation of *E. coli* K12 at UV dose of 707.2 mJ/cm$^2$. The highest inactivation of *E. coli* K12 (2.0 ± 0.1 $\log_{10}$ CFU/mL and 2.0 ± 0.4 $\log_{10}$ CFU/mL) was achieved when the cloudy apple juice was treated with both 280 nm and 280/365 nm UV-LEDs. For clear apple juice the highest inactivation 4.4 $\log_{10}$ CFU/mL obtained for *E. coli* K12 was achieved using 4 lamps emitting light at 280 nm for 40 min exposure time. For the same treatment time, the experiments using a combination of lamps emitting light at 280 and 365 nm (2lamp/2lamp) were resulted in 3.9 ± 0.2 $\log_{10}$ CFU/mL reductions. UV-A and UV-C rays in combination showed a better inactivation effect on PPO than UV-C rays used separately. Residual activity of PPO in CAJ was reduced to 32.58% when treated with UV-LED in combination of UV-C (280 nm) and UV-A (365 nm) rays. Additionally, the total color change (ΔE) of CAJ subjected to combined UV-LED irradiation at 280/365 nm was the lowest compared to other studied processing conditions. This study provides key implications for the future application of UV-LEDs to fruit juice pasteurization.

## 1. Introduction

As an alternative to consumption of fruits, drinking fruit juices is easy to consume especially for very young, elderly and infirm people. It is known that consumption of fruit juices decreases the risk of chronic diseases, retards Alzheimer disease onset, slows down LDL oxidation, inhibits platelet aggregation, and prevents the development and progression of coronary artery diseases due to their antioxidant compounds (Borenstein et al., 2005; Keevil et al., 2000; Stein et al., 1999).

Fruit juices are also susceptible to microbial spoilage though they have acidic pH values. Observation of outbreaks caused by the consumption of unpasteurized fruit juices raised a question about the safety of acidic juices. The most common microorganisms found in fruit juices are acid-tolerant bacteria, yeasts and molds. However, *Salmonella* and *Escherichia coli* 0157:H7 outbreaks indicated the potential of fruit juices to carry pathogenic microorganisms (Cook et al., 1998). Foley et al. (2002) estimated the number of cases of illnesses associated with unpasteurized juices as 16,000 to 48,000 in a year.

Thermal pasteurization is the best known technique in order to remove pathogens, reduce the number of spoilage microorganisms and inactivate the enzymes which decrease the quality of the products. However, use of high temperatures may cause some quality problems such as color, taste and flavor defects (Aguilar-Rosas et al., 2007; Choi and Nielsen, 2004).

Increased trend towards fresh-like products forced the researchers to investigate alternative processing techniques (Basaran-Akgul et al., 2009; Tahiri et al., 2006). One of the alternative method to thermal pasteurization is UV-C radiation. Antimicrobial effect of UV-C light is very well known and this technique is used for disinfection of fruits surfaces, hospital equipment, water resources etc. (Begum et al., 2009; Bintsis et al., 2000; Nigro et al., 1998; Pan et al., 2004). Inactivation mechanism of UV-C irradiation is based on the absorption of UV photons by the genetic material and the formation of dimers which inhibit the transcription and replication of the cell (Bolton and Linden, 2003; Koutchma, 2009; Oguma et al., 2002).

On the other hand, many microorganisms are able to repair damages on their DNA caused by UV-C irradiation by means of two different mechanisms depending on the light availability such as

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photoreactivation and dark-repair (Chevremont et al., 2012a; Oguma et al., 2002).

Generally low or medium pressure mercury vapor lamps are used for UV-C irradiation, first one emitting predominantly monochromatic UV irradiation at 254 nm and second one emitting polychromatic UV irradiation in the wavelengths range from 200 to 400 nm. However, these lamps contain mercury which is known to have toxic effect for both environment and human body (Mori et al., 2007). Additionally, UV reactors are required to be designed according to the shape of the lamps. These lamps are mostly large in size and take up too much space (Chevremont et al., 2012a). Moreover, UV lamps are not resistant to shock and have a low life span (approximately 4000–10,000 h). Therefore, it is necessary to design new disinfection equipment in various sizes which do not contain toxic substances and have low energy consumption rate (Crawford et al., 2005; Hamamoto et al., 2007).

In that regard, the UV disinfection using LEDs (Light Emitting Diode) seems a promising technology. UV-LEDs are created by connecting p-type and n-type semiconductors that move electrons into positively charged holes between these two materials. Light is generated when the electrons and holes recombine at a junction. The wavelength of light depends on the type of material that is used for those semiconductors (i.e. indium gallium nitride for light in the visible range, and aluminum gallium nitride and aluminum nitride for UV range) (Bowker et al., 2011). They have a very compact, shock-resistant and robust design. They can also be used for disinfection of narrow spaces and allow saving space due to their small sizes. UV-LEDs do not need a warm-up time in contrast to traditional UV-C lamps. Hence, they consume less energy. It was also reported that UV-LEDs have relatively longer life time exceeding 100,000 h (Chevremont et al., 2012a). Most importantly, they do not contain any toxic substances which are harmful for human health and the environment. They are capable of emitting UV light at multiple individual wavelengths. Besides, it is possible to use the combination of different UV-LEDs emitting light at different wavelengths.

UV-LEDs emitting light in food industry are used for three main areas such as food production, postharvest storage and food safety (D'Souza et al., 2015). A serious number of studies have published showing the efficiency of LEDs on postharvest applications succeeded in delaying of senescence in vegetables (Braidot et al., 2014; Ma et al., 2014), accelerating secondary metabolites during ripening process (Xu et al., 2014a; Xu et al., 2014b) and increase or delaying loss of postharvest nutritional content of plant parts, including edible flowers and fruits such as broccoli, citrus and strawberries (Dhakal and Baek, 2014; Ma et al., 2014; Shi et al., 2014).

There are limited numbers of studies related to the use of UV-LEDs for water disinfection. Chatterley and Linden (2010) reported that most of those data were available for LEDs emitting light at UV-A range. However, UV-C LEDs were also indicated to be preferred for this purpose (Bowker et al., 2011; Chevremont et al., 2012a; Chevremont et al., 2012b; Hamamoto et al., 2007; Li et al., 2010; Würtele et al., 2011). Moreover, combination of UV-A and UV-C LEDs was used in some studies (Aoyagi et al., 2011; Chevremont et al., 2012a). Bowker et al. (2011) indicated that emitted UV light at 275 nm resulted in much higher microbial inactivation. This is due to the fact that protein absorption spectrum reaches the maximum near 280 nm and thus, enzymes become more sensitive to inactivation at these wavelengths. Moreover, at a wavelength range of 200 to 280 nm (UVC) and 280 to 315 nm (UV-B), it has a damaging effect on DNA replication and transcription. Direct exposure to UVC or UV-B results in dipyrimidine dimers, pyrimidine hydrates, or cross-links between proteins and DNA. Hence, it is capable of inactivating a variety of pathogens such as bacteria, viruses, fungi, protozoa, and other pathogenic organisms (Lui et al., 2014). Furthermore, it is known that UV-A radiation mechanism is based on the inactivation of microorganisms by damaging proteins and producing hydroxyl and oxygen radicals which destroy cell membrane and other cellular components (Chevremont et al., 2012a). Although DNA damage caused by UV-C radiation can be repaired by the enzyme photolyase, there is no possibility to repair the damage to bacterial membranes by UV-A radiation. Chevremont et al. (2012a) showed that coupling UV-A and UV-C could be paired by using the germicidal effect of UV-C and greater penetrating ability of UV-A. They also found that use of coupled wavelengths 280/365 nm and 280/405 nm caused total disappearance of fecal enterococci, total coliforms and fecal coliforms in the effluent. Besides lack of possibility to repair the damage in the bacterial membranes occurred after UV-A exposure increased the efficiency of microbial inactivation (Chevremont et al., 2012a).

Moreover, inactivation of the quality degrading enzymes which depends on the intensity of the radiation is successfully achieved after exposure to light emitted in the range of 250–740 nm (Falguera et al., 2012). However, there is no study in the literature related to the assessment of the effect of UV-LEDs on the inactivation of microorganisms and enzymes, and quality of the fruit juices.

Therefore, the objective of this study is to investigate the applicability of UV LEDs for the non-thermal treatment of clear (AJ) and cloudy apple juice (CAJ) for the inactivation of microorganisms and quality degrading enzymes without affecting the juice quality. For this purpose, a UV-LED device was constructed for static tests and two different UV-C LED wavelengths (254 nm, 280 nm) were tested independently. UV-LEDs emitting lights in the UV-A range (365 nm) and a LED array (405 nm) were also coupled as follows: 254/365, 254/405, 280/365, 280/405 and 254/280/365/405 nm. The microbial effectiveness of UV light treatment was tested by inoculating *E. coli* K12 into AJ and CAJ. Moreover, the effect of UV-LED irradiations on the activity of polyphenol oxidase (PPO) enzyme in CAJ was investigated.

## 2. Materials and methods

### 2.1. Apple juice

Commercial pasteurized clear apple juice (AJ) (Dimes, Kemalpaşa, IZMIR) was purchased from a local market in Izmir, Turkey. AJ does not contain any citric acid and other preservatives. Background flora of pasteurized samples was tested by surface plating on Plate Count Agar (PCA, Sigma-Aldrich, St. Lous, MO, USA) for enumeration of total aerobic bacteria and Violet Red Bile Agar (VRBA, Merck, Darmstadt, Germany) to determine the number of coliforms prior to UV treatment.

For the production of fresh CAJ, apples of the Starking Delicious variety were purchased from a local supermarket (Tesco-Kipa) in Izmir, Turkey. Apples were washed under tap water and patted dry and squeezed using a household table top juice extractor (Arçelik Robolio, İstanbul). After the extraction step, the juice was strained through a sterile gauze strip to remove the big particles and foams in the juice.

### 2.2. Physicochemical and optical properties

The total soluble solids (TSS) and pH of fresh CAJ and AJ were measured using a bench top refractometer (Mettler-Toledo RE40D, AEA Investors Inc., USA) and a benchtop pH meter (HANNA Instruments, USA). The titratable acidity of samples was determined according to the method of AOAC (1990) and expressed as the weight of malic acid in 100 mL (w/v).

Turbidity was determined using a turbidimeter (Model 2100AN IS, HACH Company, USA) and expressed in Nephelometric Turbidity Units (NTU). Absorbance coefficients of the juice samples were determined using a 1 cm quartz cuvette in a Cary 100 UV–Visible Spectrophotometer (Varian, USA) adjusted to 254 and 280 nm. A variety of dilution factors were applied (1:10, 1:25, 1:50, 1:100, 1:250, 1:500). The absorption coefficient ($cm^{-1}$) at each individual wavelength was estimated by the slope of absorbance versus sample concentration plot (Hakgüder, 2009).

The color parameters were determined by means of a Konica