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UV-C irradiation as an alternative disinfection technique: Study of its effect on polyphenols and antioxidant activity of apple juice

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(−)-Epicatechin (PubChem CID: 72276)
D(−)-Fructose (PubChem CID: 5984)
D-Glucose (PubChem CID: 5793)

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ABSTRACT

Ultraviolet (UV-C) irradiation is a non-thermal disinfection method, effective against a range of bacteria and viruses, which is being considered as an alternative to pasteurization of fruit juices. The objective of this study was to investigate the effect of UV-C irradiation on the polyphenolic content and *in-vitro* total antioxidant activity of apple juice. UV irradiation doses ranging from 0 to 240 mJ·cm^{-2} were delivered to apple juice and polyphenols, sugars, *in-vitro* total antioxidant activity and total phenols were profiled. The results demonstrated that UV-C irradiation in apple juices at relevant commercial disinfection doses induced significant reduction in the concentrations of chlorogenic acid, phloridzin, and epicatechin ($p < 0.05$). The induced changes were relatively minor for the above mentioned polyphenols, except phloridzin (50% reduction) at 240 mJ·cm^{-2}. Epicatechin concentrations were reduced significantly ($p < 0.05$), whereas increase in catechin concentration was observed with increase in UV-C exposure to 240 mJ·cm^{-2}. There was a minor reduction in sugar (glucose and fructose) concentrations with increasing exposure levels from 0 to 40 mJ·cm^{-2} ($p > 0.05$). In contrast, a slight increase in sugar concentrations as increase in UV-C exposure after 40 mJ·cm^{-2} was observed. These changes were not significantly different from control. Total phenolic content was well retained regardless of the UV-C exposure for apple juice. *In-vitro* total antioxidant activity changed when UV-C exposure exceeded 40 mJ·cm^{-2}, but remained unchanged at the maximum UV-C dose of 240 mJ·cm^{-2}. These results suggested that UV-C irradiation could be an effective alternative to conventional thermal processing for production of high quality apple juice.
Industrial Relevance: This research paper provides scientific evidence of the potential for UV-C irradiation to achieve meaningful levels of disinfection while retaining important bioactive compounds (polyphenols) in apple juice. *In-vitro* antioxidant activity and individual polyphenols were well retained at commercially relevant doses of 40 mJ·cm^{-2}. From a nutritional perspective, UV-C irradiation is an attractive food preservation technology and offers opportunities for horticultural and food processing industries to meet the growing demand from consumers for healthier food products. Therefore, UV-C irradiated foods could be sold at a premium price to their thermally-processed counterparts, as they have retained their fresh-like properties. This study would provide technical information relevant for commercialization of UV-C treatment of juices.

1. Introduction

Apples and apple based products are excellent sources of some important phytochemicals, including several polyphenols responsible for their antioxidant properties (Kahle, Kraus, & Richling, 2005; Khanizadeh et al., 2008; Van der Sluis, Dekker, Skrede, & Jongen, 2002). Phenolic compounds are known as scavengers of active oxygen and other free radical species (Fernandez-Panchon, Villano, Troncoso, & Garcia-Parrilla, 2008; Fukumoto and Mazza, 2000) and exhibit a strong preventive role against some major diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and obesity (Boyer & Liu, 2004; Hollman, 2001; Ren, Qian, Wang, Zhu, & Zhang, 2003; Spencer, 2010). In apple juice, two major polyphenols, chlorogenic acid and phloridzin, along with catechins (Eisele & Drake, 2005) have been recognized for a wide range of biological functionality beneficial to human health (Shin et al., 2015; Gonzalez-Gallego, Garcia-Mediavilla, Sanchez-Campos, & Tunon, 2010; Milani et al., 2015; Zaveri, 2006; Zhao et al., 2004). Due to the presence of various

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polyphenols and other vitamins, apples have been extensively investigated as an important source of antioxidants (Boyer & Liu, 2004).

Thermal processing, which is utilized to inactivate pathogens and spoilage organisms, can reduce the concentrations of polyphenols and other nutrients in foods. Depending on the food processing techniques, stability of polyphenols varies (Ioannoua, Hafsaa, Hamdib, Charbonnela and Ghoula, 2012; Rawson, Patras, Tiwari, Koutchma and Brunton, 2011a). Thermal technologies (pasteurization, sterilization, hot air drying etc.) can bring chemical and physical changes that can impact organoleptic properties and can reduce the content or bioavailability of bioactive compounds (Patras, Brunton, Butler, & Downey, 2009a; Patras, Brunton, Tiwari, & Butler, 2009b; Patras, Brunton, O'Donnell, & Tiwari, 2010; Rawson, Hossain, Patras, Tuohy and Brunton, 2011b). In order to avoid the detrimental effect of thermal processing techniques on liquid foods, non-thermal disinfection techniques have been investigated over the last few years with the objective of developing products with unique functionality and improved quality (Rawson et al., 2011a).

Since the United States Food and Drug Administration (FDA) approval of the use of UV light as a novel technology for pasteurization of fruit juices (USDA, 2000), recent advances in science and engineering have clearly demonstrated that UV technology holds considerable promise as an alternative to traditional thermal processes such as pasteurization for food preservation (Worobo, 1999; Hanes et al., 2002; Matak et al., 2005). However, many of these published research studies are empirical in nature and the documented results are quite difficult to generalize and apply to different contexts and applications. Therefore, the application of UV light to opaque liquid foods was previously considered impractical if not impossible. Only recently, as more detailed approaches to UV disinfection of food products have been proposed (Koutchma, Forney, Moraru, & Sun, 2009), this application has shown larger, and practical promise to the food and beverage industries.

Recent studies demonstrate the efficacy of UV technology for treating fruit juices. Not all liquid foods can tolerate the pasteurization process, as they contain heat sensitive bio-molecules such as vitamins, polyphenols, caroteniods, glucosinolates, and amino acids. As a non-thermal process, UV treatment may hold promise for these products. Recent bench scale studies have shown that UV-C technology may be used to preserve liquid food products including: fruit juices such as orange juice (Tran & Farid, 2004; Keyser, Műller, Cilliers, Nel, & Gouws, 2008), apple juice (Keyser et al., 2008; Franz, Specht, Cho, Graef, & Stah, 2009; Caminiti et al., 2012), pineapple juices (Chia, Rosnah, Noranizan, & Wan Ramli, 2012), grape, cranberry and grapefruit juices (Guerrero-Beltrán, Velti-Chanes, & Barbosa-Cávanos, 2009), pomegranate juice (Pala & Toklucu, 2011), and liquid egg white (Unluturk, Atilgan, Baysal, & Unluturk, 2010).

Because of increasing awareness among consumers, the demand for minimally-processed foods has increased dramatically, driving the development and application of non-thermal technologies for disinfecting liquid foods. Compared with thermal treatments, non-thermal treatments can have less destructive effects on nutritional and sensory properties of foods. Therefore, the introduction of a new processing method that could conserve the nutritional quality of products would be highly desirable.

Significant gaps remain on the impact of UV treatment on the functional attributes of liquid foods, including apple juice. The effects of UV irradiation on the individual polyphenols and *in-vitro* antioxidant activity content of apple juice has not been reported to date; however, UV irradiation was reported to have minor effects on the flavonoids (Alothman, Bhat, & Karim, 2009a, 2009b).

A common weakness in many UV irradiation studies is that they do not consider the optical absorbance of the fluid (Unluturk et al., 2010; Caminiti et al., 2012). The present study investigates the effect of UV-C irradiation on the individual polyphenolic content and *in-vitro* total antioxidant activity of apple juice. The optics of the fluid are accounted for, and dose delivery is verified through bio-dosimetry, ensuring that target levels of disinfection are achieved, and allowing direct comparisons with other UV treatment studies.

2. Materials and methods

2.1. Chemicals

Phloridzin dehydrate, fructose, glucose, gallic acid, trifluoroacetic acid (TFA), acetonitrile, 2,2-diphenyl-1-picrylhydrazyl, sodium carbonate, and Folin–Ciocalteu reagent were purchased from Sigma Aldrich, USA. Chlorogenic acid was purchased from Extrasynthese, France. Ascorbic Acid was sourced from Alfa Aesar, USA. (+)-Catechin and (−)-epicatechin were purchased from Adooq Bioscience, Irvine, CA, USA.

2.2. Juice preparation

Fresh apple juice was purchased from a local supplier (Nashville, TN, USA). The juice was filtered using Whatman filters (28–30 μm, 2–3 μm) and stored at −20 °C wrapped with aluminum foil until further processing, to avoid exposure to light. Two consecutive filtration steps were carried out to remove solid particulates. Prior to irradiation, apple juice samples were randomly assigned to a treatment process and thawed at room temperature.

2.3. UV irradiation treatments

Irradiations were performed using a "Collimated Beam" device incorporating a low-pressure mercury lamp emitting at 254 nm. This apparatus was designed to provide uniform, quantified irradiation to liquid samples, and the associated methods, including calibration, fluence determination, and quality assurance protocols, have been developed and standardized in the field of water disinfection (Bolton & Linden, 2003). To enhance mixing, the irradiated volumes were reduced to 5 mL samples in 10 mL beakers, with continuous stirring. UV fluence values were calculated using the standard method based on measured UV irradiance and optical properties of the fluid.

The average UV fluence rate in the stirred sample can be calculated as

$$E'avg = E_0 \times P_f \times (1-R) \times \frac{L}{d+L} \times \frac{1-10^{-Ad}}{Ad \ln(10)} \quad (1)$$

where E_0 is the radiometer reading at the center of the dish and at a vertical position so that the calibration plane of the detector head is at the same level as the top of the solution. The average germicidal fluence (UV dose) ($mJ \cdot cm^{-2}$) is then given by the product of $E'avg$ and the exposure time t (secs). P_f is termed the Petri factor defined as the ratio of the UV intensity measured at the center of the sample surface to the average intensity measured across the sample surface, 1-R is termed the reflection factor where R is the fraction of UV light at 253.7 nm reflected at the air-surface interface (typically $R = 0.025$), L/(d + L) is termed the divergence factor where L is the distance from the lamp centerline to the sample surface and d is the sample depth, and $(1–10^{-Ad})/(Ad \ln(10))$ is termed the absorbance factor where A is the UV absorbance coefficient ($base_{10}$) at 254 nm of the fluid. The irradiance at the sample location was measured using an International Light Technologies (Peabody, MA, USA), IL-1700 radiometer with an SED 240 detector and a NS254 filter. The radiometer and detector were calibrated by International Light and are accompanied by NIST traceable certifications (Bolton & Linden, 2003).

2.4. Verification of UV fluence

In order to determine the actual UV fluence values delivered to the apple juice, a viral clearance test was conducted using a challenge