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

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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 $\text{mJ}\cdot\text{cm}^{-2}$ 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 $\text{mJ}\cdot\text{cm}^{-2}$. Epicatechins concentrations were reduced significantly ($p<0.05$), whereas increase in catechins concentration was observed with increase in UV-C exposure to 240 $\text{mJ}\cdot\text{cm}^{-2}$. There was a minor reduction in sugars (glucose and fructose) concentrations with increasing exposure levels from 0-40 $\text{mJ}\cdot\text{cm}^{-2}$ ($p>0.05$). In contrast, a slight increase in sugars concentrations as increase in UV exposure after 40 $\text{mJ}\cdot\text{cm}^{-2}$ was observed. These changes were not significantly different from control. Total phenolic content was well retained regardless of the UV exposure for apple juice. *In-vitro* total antioxidant activity changed when UV exposure exceeded 40 $\text{mJ}\cdot\text{cm}^{-2}$, but remained unchanged at the maximum UV dose of 240 $\text{mJ}\cdot\text{cm}^{-2}$. 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.

Chemical compounds studied in this article

Chlorogenic acid (PubChem CID: 1794427); Phloridzin (PubChem CID: 9912668); (+)-Catechin (PubChem CID: 9064); (-)-Epicatechin (PubChem CID: 72276); D(-)-Fructose (PubChem CID: 5984); D-Glucose (PubChem CID: 5793).

Keywords: UV-C Irradiation, antioxidant, polyphenols, sugars, apple juice

1. Introduction:

Apples and apple based products are excellent sources of some important phytochemicals, including several polyphenols responsible for their antioxidant properties (Kahle et al., 2005; Khanizadeh et al., 2008; Van der Sluis et al., 2002). Phenolic compounds are known as scavengers of active oxygen and other free radical species (Fernandez-Panchon et al., 2008; Fukumoto et al., 2000) and exhibit a strong preventive role against some major diseases,

84 including cardiovascular diseases, cancers, neurodegenerative diseases, diabetes, and obesity.(
85 Boyer & Liu, 2004; Hollman, 2001; Ren et al., 2003; Spencer, 2010). In apple juice, two major
86 polyphenols, chlorogenic acid and phloridzin, along with catechins (Eiselea & Drake, 2005) have
87 been recognized for a wide range of biological functionality beneficial to human health (Shin et
88 al., 2015; Gonzalez-Gallego et al., 2010; Milani et al., 2015; Zaveri, 2006; Zhao et al., 2004).
89 Due to the presence of various polyphenols and other vitamins, apples have been extensively
90 investigated as an important source of antioxidants (Boyer & Liu, 2004).

91 Thermal processing, which is utilized to inactivate pathogens and spoilage organisms, can reduce
92 the concentrations of polyphenols and other nutrients in foods. Depending on the food processing
93 techniques, stability of polyphenols varies (Ioannou et al., 2012; Rawson et al., 2011a). Thermal
94 technologies (pasteurization, sterilization, hot air drying etc.) can bring chemical and physical
95 changes that can impact organoleptic properties and can reduce the content or bioavailability of
96 bioactive compounds (Patras et al., 2009a, b; Patras et al., 2010; Rawson et al., 2010; Rawson et
97 al., 2011b). In order to avoid the detrimental effect of thermal processing techniques on liquid
98 foods, non-thermal disinfection techniques have been investigated over the last few years with
99 the objective of developing products with unique functionality and improved quality (Rawson et
100 al., 2011a).

101 Since the United States Food and Drug Administration (FDA) approval of the use of UV light as
102 a novel technology for pasteurization of fruit juices (USDA, 2000), recent advances in science
103 and engineering have clearly demonstrated that UV technology holds considerable promise as an
104 alternative to traditional thermal processes such as pasteurization for food preservation (Worobo
105 1999; Hanes et al., 2002; Matak et al., 2005). However, many of these published research studies
106 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 et al., 2009), has this application 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, carotenoids, 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 et al., 2008), apple juice (Keyser et al., 2008; Franz et al., 2009; Caminiti et al., 2012), pineapple juices (Chia et al., 2012), grape, cranberry and grapefruit juices (Guerrero–Beltrán et al., 2009), pomegranate juice (Pala & Toklucu, 2011), and liquid egg white (Unluturk et al., 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 (Allothman et al., 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 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 ciocalteau 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

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

Equation. 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) ($\text{mJ} \cdot \text{cm}^{-2}$) 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 $(1 - 10^{-Ad})$

$^{Ad})/(Ad \ln(10))$ is termed the absorbance factor where A is the UV absorbance coefficient (base₁₀) 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 organism. The challenge organism, MS2, is a single stranded RNA virus, with an icosahedral shell approximately 27 nm in diameter, used extensively in validation of UV disinfection systems for drinking water (Pirnie et al., 2006).

The UV sensitivity of the MS2 was first established using an inactivation test. MS2 bacteriophage was suspended in phosphate buffered saline and irradiated in a collimated beam to various UV fluence values. The buffer used in this characterization had high transmittance at 254 nm, typically 90% at 1 cm, so that the UV intensity gradient in the fluid was small, and therefore the uncertainty in the UV fluence was small. The 254 nm irradiance at the sample surface was measured using a radiometer with NIST-traceable calibration. The UV fluence gradient caused by optical absorbance of the fluid, along with the effects of beam divergence, non-uniformity, and surface reflection were all accounted for as recommended in the standard method. The samples were stirred during irradiation to ensure that all organisms were exposed to the same integrated UV fluence over the course of the irradiation. After irradiation, each sample was diluted serially and aliquoted into culture tubes containing 1 mL *Escherichia coli* broth culture and 20 mL of molten tryptone yeast extract glucose agar containing triphenyl tetrazolium

chloride. The mixtures were mixed by inversion and plated into sterile Petri dishes. The agar was allowed to solidify and the plates were incubated at 35 ± 0.5 °C for 18–24 h before performing a plaque assay. The plates were then evaluated to determine the number of plaque forming units. By comparing with the control (non-irradiated) sample, the relationship between UV dose and the log-reduction of this population of MS2 was established.

With the UV-sensitivity of the organism determined, the organism was then used to quantify the UV dose delivered to a UV-absorbing fluid. Ideally, the MS2 would be inoculated into the juice, followed by UV irradiation. However, the MS2 would be inactivated by the low pH of apple juice. For this reason, a surrogate fluid with the same optical absorbance and near-neutral pH was used to verify UV dose delivery. UV dose applied to the surrogate fluid was determined by using MS2 as the dose indicator. This is sometimes denoted as the reduction equivalent dose (RED) or fluence, since it is inferred from the log reduction of a well-characterized challenge organism. MS2 from the characterized population was inoculated into a surrogate fluid consisting of an aqueous solution of humic acid formulated to have the same optical absorbance as apple juice. The optical properties of the inoculated organic humic acid and the juice were measured using a spectrophotometer connected to a single integrating sphere. The optical properties of the organic humic acid were used to calculate the irradiation times necessary to achieve a desired UV dose in the collimated beam apparatus. The samples were then irradiated for the prescribed time, and then serially diluted and cultured as described above. The log reduction in numbers of active MS2 was used to calculate the UV dose received by the organic humic acid in each irradiation by using the sensitivity of the MS2 as established by the buffer tests. The resulting reduction equivalent dose vs. target dose has been plotted and may be seen in Figure 1. This relationship was used to determine the UV fluence values used in subsequent

irradiations of apple juice. This test confirmed that doses from 0- 150 mJ·cm⁻² can be applied to apple juice.

2.6 HPLC-PDA and HPLC-MS/MS method for polyphenols

The quantitation of chlorogenic acid and phloridzin was carried out on a Shimadzu Prominence XR UHPLC (Shimadzu Scientific Instruments, Columbia, MD, USA) which included two Shimadzu LC-20ADXR pumps, a SIL-20ACXR autosampler, a CTO-20A column oven, and a SPD-M20A photo diode array (PDA) UV-VIS detector. Chromatographic separation was achieved on with a Phenomenex Kinetex 2.6 C18 column (50 × 2.1 mm, 2.5 µm) maintained at a temperature of 50 °C. The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water (A) and 0.1% TFA in acetonitrile (B). The following gradient program was used: 0 to 1.1 min, 3% B; 4.0 min, 30% B; 4.10 min, 100% B; 4.21 min, 100% B. Finally, solvent B was returned to 3% and remained at 3% until the end of the program at 6.5 min. The flow rate was maintained at 0.75 mL min⁻¹. The injection volume was 10 µL. The PDA was set to acquire absorbance from 190 to 350 nm. The UHPLC system was also interfaced to a Shimadzu LCMS 2020 single quadrupole mass spectrometer for identification of chromatographic peaks. An electrospray ionization source in the positive ion mode was utilized with the following parameters: DL temperature 250 °C, nebulizing gas flow, 1.50 L min⁻¹, heat block 500 °C, and drying gas flow, 15 L min⁻¹. The scan range was 150 to 700 m/z with a scan speed of 5,000 scans per second. Quantitation was accomplished by analyzing the chromatographic peak areas of the standards and samples at 320 nm. Standard mixtures of chlorogenic acid and phloridzin were prepared (six calibrants from 3.56 to 100 µg mL⁻¹) in 0.2% acetic acid in water. Data were acquired and analyzed with Shimadzu Lab Solutions software. The peak areas of the standards were plotted on calibration curves for quantitation by linear least squares regression. The determination

coefficients (R^2) were 0.99999 for chlorogenic acid, and 0.99 for phloridizin. The quantitation of epicatechin and catechin was carried out with an LC-MS/MS method using a Shimadzu Prominence XR UHPLC interfaced to a Shimadzu LCMS 8030 triple quadrupole mass spectrometer. The chromatographic separation was the same as the chlorogenic acid and phloridizin UHPLC-UV method except the mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The injection volume was 5 μ L. The MS method was run in negative ion mode with a combined ESI-atmospheric chemical ionization source using multiple reaction monitoring (MRM) to monitor the mass transitions. The MRM transition for both catechin and epicatechin was m/z 289.0 $\rightarrow$ 109.0. The collision energy was 25 V, the DL temperature was 250 $^{\circ}$ C, the nebulizing gas flow was 1.50 L min^{-1} , the heat block was 500 $^{\circ}$ C, and the drying gas was 15 L min^{-1} . Catechin and epicatechin were quantified by comparing MRM chromatographic peaks from samples with standards. Standard mixtures of catechin and epicatechin were prepared (eight calibrants from 0.195 to 50 $\mu\text{g mL}^{-1}$) in 0.2% acetic acid in water. The calibrants were run in duplicate and the average peak areas were plotted in a linear calibration curves with 1/x weighting. R^2 was 0.99880 for epicatechin and 0.99963 for catechin.

2.7 Total Phenolic Contents (TPC)

Total phenolic content of apple juice was assessed using a modified version of the Folin–Ciocalteu assay (Singleton et al., 1999). Gallic acid was used as a standard and the aqueous gallic acid solution (100, 75, 50, 25, 10 mg L^{-1}) was diluted with distilled water to give appropriate concentrations for a standard curve. For the analysis, 100 μ L of fruit juice sample (samples were diluted in milli-Q water at 1 in 3 dilution) or gallic acid standard, 100 μ L of water, 100 μ L of Folin–Ciocalteu reagent and 700 μ L of Na_2CO_3 (20%) were added into 1.5 mL micro-centrifuge tubes. The samples were vortexed immediately and the tubes were incubated in the

dark for 20 min at room temperature. After incubation all samples were centrifuged at 10,000 g for 3 min. The absorbance of the supernatant was then measured at 740 nm in 1 mL plastic cuvettes using a spectrophotometer (GENESYS 10S UV-vis, Thermo Scientific, Waltham, MA, USA). Three replicates for each dose were analyzed and the results were expressed on 100 ml of juice in mg of gallic acid equivalent (mg GAE/100 mL).

2.8 DPPH Free Radical Scavenging Activity

The radical scavenging activity was tested according to the method described by Shimada et al. (1992) and Gordon (2001) with some necessary modifications. A 50µL apple juice aliquot was added to 1.5 mL of methanolic HCl (HPLC grade) DPPH (200 µM). The mixture was allowed to stand at darkness for 30 minutes then absorbance was measured at 517 nm using GENESYS 10S UV-vis spectrophotometer. A Standard curve ($y = -33.612x^2 + 160.57x - 0.9198$, $R^2 = 0.99$) was developed by using ascorbic acid as the positive control. The capacity to scavenge the DPPH radical was calculated using the following equation:

$$\text{Scavenging Effect (\%)} = \left[\frac{A_0 - (A - A_b)}{A_0} \right] \times 100\% \quad \text{Equation. 2}$$

where A_0 is the absorbance of DPPH solution without sample, A is the absorbance of the test sample mixed with DPPH and A_b is the absorbance of the without DPPH solution. Results were expressed as percentage of inhibition of the DPPH radical.

2.8 HPLC-ELSD Method for Sugar Quantification

Determination of fructose and glucose in controls and irradiated apple juice was carried out according to the HPLC-ELSD method described by Ma C. et al. (2014) with some minor modification. HPLC analysis was performed on a Shimadzu Prominence system equipped with

LC-20AD gradient pump, Shimadzu ELSD-LT II detector, CBM-20A communication bus module, SIL-20A HT autosampler and Shimadzu LC solution software. Separations were carried out on Phenomenex Luna 5u NH2 100A column (250 mm x 4.60 mm, 5 micron) (Phenomenex, USA). Nebulizing gas temperature was set at 40°C and gas pressure was tuned to 50 psi. Total run time was 20 mins. The mobile phase was comprised of acetonitrile: water (82.5:17.5, v/v) in isocratic mode. The flow rate was set at 1.5 mL min⁻¹ and an aliquot of 10µL was injected.

2.9 Statistical Analysis

The concentration of polyphenols, fructose, glucose, total phenolic contents and antioxidant activity were evaluated under six UV doses levels (0, 20, 40, 80, 120, 240 mJ·cm⁻²). A balanced design with three replicates for each treatment was exposed to the selected UV radiation treatment. Each sample was independent and assigned randomly to a treatment. One-way ANOVA with Tukey's HSD multiple comparison tests were performed to assess the effects of different treatments on polyphenols, antioxidant activity, total phenols and sugars in R statistical computing environment (R Development Core Team 2015). Data are presented as means ± one standard deviation from the mean. Statistical significance were tested at 5 percent significance level (p<0.05).

3. Results and Discussion:

Disinfection by UV is strongly affected by the optics of the fluid, since all fluid elements must be exposed to UV in order to achieve significant log reductions of pathogens and spoilage organisms. This oversight means that the data cannot be related to any desired disinfection result, or compared against studies of a different fluid with different optical properties (Unluturk et al.,

2010; Caminiti et al., 2012). In most cases, the authors quantified only UV incident dose at the surface of system, measuring the incident intensity at the surface of the liquid with a radiometer (Assatarakul, Churey, Manns, Worobo, 2012). In fact, the incident intensity at the surface is not the incident intensity at any other depth.

The collimated beam approach is based on using well-mixed samples in a quantifiable UV radiation field, accounting for all relevant optical factors such as beam divergence, reflection, and non-uniformity. It assumes that the mixing is sufficient to ensure that all fluid elements are exposed equally and that the geometric average UV intensity may therefore be used to calculate total fluence. This approach overcomes the problems in other studies in which stagnant, highly absorbent fluid samples were irradiated.

Six compounds were identified and quantified by HPLC-PDA-MS, HPLC-ELSD, and HPLC-MS/MS, namely chlorogenic acid, phloridzin, epicatechin, catechin, fructose, and glucose. Except for glucose, the other compounds had statistically significant trends with respect to mean concentrations as a function of UV doses ranging from 0-240 $\text{mJ}\cdot\text{cm}^{-2}$. Significant changes in *in-vitro* antioxidant activity were observed for irradiated apple juice samples after UV exposure of 40 $\text{mJ}\cdot\text{cm}^{-2}$ ($p<0.05$). In contrast, no change in total phenolic content of irradiated apple juice was found ($p>0.05$).

There is a linear relationship between target dose and reduction equivalent dose (RED) expressed as $\text{mJ}\cdot\text{cm}^{-2}$ (Figure 1). It demonstrated the dose delivery in organic humic acid which exhibits an UV transmittance of $10^{-5}\% \text{ cm}^{-1}$ and indirectly verifies the fluence in apple juice.

3.1 Polyphenols

327 A typical HPLC chromatogram for polyphenols is shown in Figure 2. Figure 3 illustrates the
328 effect of UV-C irradiation on the polyphenolic content of apple juice. Chlorogenic acid and
329 phloridzin are two abundant polyphenolic compounds present in many apple juice varieties
330 (Eiselea & Drake, 2005). Chlorogenic acid and phloridzin were detected at a concentration of
331 $4.04\text{mg}100\text{mL}^{-1}$ and $1.79\text{mg}100\text{mL}^{-1}$ respectively, in the control samples. When the
332 concentration profiles of these two compounds were compared with changes from exposure to
333 visible light and air (without UV) in the control samples, it was found that their concentrations
334 were relatively stable (data not shown). Conversely, concentrations of chlorogenic acid and
335 phloridzin were observed to almost linearly diminish with exposure to UV-C irradiation
336 ($p<0.05$), (Figure 3a and b). Both polyphenolic compounds changed in relative concentration
337 ranging from a decrease of 19.3% and 50% for chlorogenic and phloridzin, respectively, at the
338 maximum dose of $240\text{ mJ}\cdot\text{cm}^{-2}$. Results indicated phloridzin to be very sensitive to UV-C
339 irradiation. It is well established in the literature that degradation of polyphenolic compounds is
340 related to the photo-oxidation or photo-induced molecular rearrangement, which is greatly
341 influenced by many factors including pH, the presence of oxygen, wavelength of light, structure
342 and concentration of phytochemicals, and the interaction among them in the medium.
343 (Bordignon-Luiz et al., 2007; Aramwit et al., 2010; Março et al., 2011; Ioannou et al., 2012;
344 Tommasini et al. 2004).

345 Riboflavin was detected in control apple juice samples (data not shown). It has a complex
346 photochemistry because of its ability to accept or lose a pair of hydrogen atoms (Kim, H.,
347 Kirschenbaum, Rosenthal, & Riesz (1993). Riboflavin under light can generate reactive oxygen
348 species such as superoxide anions and singlet oxygen (Kumari and others 1996; Grzelak and
349 others 2001; Min and Boff 2002; Mahns and others 2003). Formation of reactive oxygen species

by vitamins (especially riboflavin) under light proceeds by both Type-I and Type-II mechanisms (de La Rochette and others 2003). In the Type-II mechanism, the excited triplet riboflavin may react with triplet oxygen to produce superoxide anion or singlet oxygen (Foote 1985). Huang, Choe, and Min (2004) reported that riboflavin produces singlet oxygen from triplet oxygen under light. The authors reported that riboflavin under normal light produces singlet oxygen and the singlet oxygen reacts. Reactive oxygen species may have degraded chlorogenic and phloridzin in irradiated apple juice (Figure 3a and b).

Concentrations of catechin and (-)-epicatechin were also observed to change with exposure to UV-C irradiation (Figure 3). (-)-Epicatechin content decreased significantly ($p < 0.05$) as a function of UV dose, in contrast to catechin which showed an absolute increase ($p < 0.05$). The increase in catechin concentration at higher doses of UV-C irradiation may occur from the conversion of (-)-epicatechin to its corresponding isomer, (-)-catechin. The epimerization of catechins in aqueous solutions has been shown to occur at pH levels over 6 or temperatures over 40 °C (Wang & Helliwell, 2000; Komatsu et al, 1993). Heat induced epimerization occurs at the 2-position via a ring opening reaction and recyclization that favors the trans-form (Mehta & Whalley, 1963). We propose that UV-C irradiation induces the epimerization of (-)-epicatechin to (-)-catechin in a manner similar to heat induced-isomerization. The percentage of epicatechin converted to catechin does not total 100% due to other reactions that take place such as oxidation and degradation (Seto et al. 1997). It should be noted that, using the stated HPLC-LC/MS/MS method, (-)-catechin and (+)-catechin cannot be separated; however, the increase in the catechin concentration can be assumed to be due to (-)-catechin formation from (-)-epicatechin (Wang & Helliwell, 2000). The UV doses applied in this study are greater than those required for significant disinfection of many organisms. The FDA has approved UV light as an alternative

treatment to thermal pasteurization of juice products such as apple juice (USDA, 2000). Extensive research on the application of UV light for fresh apple cider and apple juice by Worobo (1999) recommended that the process be designed to deliver at least 5-log reduction of *E. coli O157:H7*. The FDA has documented that UV radiant exposure must be at least $40 \text{ mJ} \cdot \text{cm}^{-2}$ at all part of the products to achieve sufficient microbial disinfection. At the dose threshold set by the FDA ($40 \text{ mJ} \cdot \text{cm}^{-2}$) the UV treatment resulted in minor but significant changes ($p < 0.05$) in polyphenols, except phloridin, where 20% reduction was observed ($p < 0.05$).

3.2 *In-vitro* antioxidant activity and total phenolic content

In-vitro antioxidant activity and total phenolic content were assessed spectrophotometrically using DPPH and Folin–Ciocalteu assay, respectively. The total phenol content was found to be relatively stable and ranged from 9.79 to $9.48 \text{ mg GAE } 100 \text{ mL}^{-1}$ (figure 4) in control and irradiated apple juice samples, therefore UV irradiation did not induce significant changes ($p > 0.05$), (Figure 4a). *In-vitro* total antioxidant activity (DPPH scavenging activity) of control and irradiated apple juice ranged from 18.94% to 16.49%; therefore, only minor changes were observed over the UV dose range of $0\text{--}40 \text{ mJ} \cdot \text{cm}^{-2}$. There is a significant change in *in-vitro* antioxidant activity at a dose of $40 \text{ mJ} \cdot \text{cm}^{-2}$ compared to control, however there were no significant changes with further increases in UV dose (Figure 4). That is, increasing dose from 40 to $240 \text{ mJ} \cdot \text{cm}^{-2}$ did not result in a significant change in antioxidant activity ($p > 0.05$).

3.3 Sugars

Most fruit juices contain varying quantities of sugars, such as fructose, glucose, sucrose, and maltose (Karadeniz & Eksi, 2002). Fructose and glucose were the two major sugars quantified in control and irradiated apple juice. The concentration of fructose and glucose in control samples

was 8759.59 mg100 mL⁻¹ and 2662.70 mg100 mL⁻¹ respectively. Concentrations of both sugars were observed to change slightly with exposure to UV-C irradiation. For fructose, no significant differences were observed between controls and UV irradiated samples. However, samples irradiated at low UV doses (20 & 40 mJ·cm⁻²) were significantly different from samples irradiated at 240 mJ·cm⁻² (p<0.05). Similar trend was observed for glucose [Figure 5a and b]. Even at high UV doses applied (80- 240 mJ·cm⁻²) in this study, concentration of fructose and glucose were relatively stable.

4. Conclusion

Commercially relevant UV doses utilized in this study induced minor changes in *in-vitro* total antioxidant activity, polyphenolic content, and sugar content of irradiated apple juice. At the FDA-recommended UV Dose of 40 mJ·cm⁻², phloridzin concentration was significantly reduced in apple juice, and the level of reduction was substantially higher at higher exposure levels. The concentration of epicatechins was significantly decreased as a function of UV dose, whereas an absolute increase in catechins concentration was observed. These changes were more pronounced after 40 mJ·cm⁻², i.e. FDA suggested dose delivery threshold for apple juice treatment. The findings from this study serve as a demonstration of some of the changes that can occur to individual polyphenols, antioxidant activity and sugars after applying UV doses between 0 and 240 mJ·cm⁻². While these results may not be generalizable to all juices, they provide insights into the effects of UV treatment of fruit juice, and identify compounds of interest for further study. In addition, investigations into the impact of UV treatment on cell toxicity and sensory quality in UV-treated fruit juices is warranted in the future studies.

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Figure 1. Received UV fluence in organic humic acid measured by bioassay (MS2 bacteriophage) vs. target value. The reduction equivalent dose (RED) applied to organic humic acid was determined by well-characterized MS2 phage as the dose indicator (Target dose).

Figure 2. Polyphenol sample chromatogram, [A] 320 nm chlorogenic acid peak at 2.752 min, [B] UV 280 nm phloridzin peak at 4.17 min and [C] LC-MS/MS multiple reaction monitoring (MRM) chromatogram of catechin (1.649 min) and epicatechin (2.149 min).

Figure 3. Effect of UV-C irradiation on polyphenols (mean value $\pm$ SD, n = 3) in the apple juice: a) chlorogenic acid, b) phloridzin, c) epicatechin and d) catechin. In the same column; figures with different letters are different at a statistical level of $p < 0.05$

Figure 4. Effect of UV-C irradiation on total phenolic content (mean value $\pm$ SD, n = 3) and antioxidant activity (mean value $\pm$ SD, n = 3) in apple juice; figures with different letters are different at a statistical level of $p < 0.05$

Figure 5. Effect of UV-C irradiation on fructose (mean value $\pm$ SD, n = 3) and glucose (mean value $\pm$ SD, n = 3) in apple juice; figures with different letters are different at a statistical level of $p < 0.05$

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.