UV-C light processing of *Cantaloupe* melon juice: Evaluation of the impact on microbiological, and some quality characteristics, during refrigerated storage

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Abstract

UV-C light is a non-thermal technology with potential application in food industries. The objective was to assess UV-C radiation effect (13.44 W/m^2) on microbiological decontamination and some quality characteristics of *Cantaloupe* melon juice, during refrigerated storage.

Juice was inoculated with *Listeria innocua* (non-pathogenic surrogate of *L. monocytogenes*) and *Alicyclobacillus acidoterrestris* spores (spoilage indicator) used as indicators of the UV-C treatment efficacy. Total mesophylls and yeasts and molds were also enumerated. Results demonstrated that 5 minutes of exposure allowed 3.7±0.3 log_{10} cycles reduction of *L. innocua*, while for *A. acidoterrestris*, 20 minutes were required to decrease 4.7±0.1 log_{10} cycles.

At the end of refrigerated storage for 13 days, UV-C treated juices retained color, total phenolics content and antioxidant activity, and yeast and molds did not grow.

Since UV-C process was effective on microbial inactivation and allowed juice quality maintenance, it can be considered as a promising alternative to thermal pasteurization.

Keywords: melon juice, UV-C radiation, *L. innocua*, *A. acidoterrestris*, storage

1. Introduction

Cantaloupe melon (*Cucumis melo* L. var. *reticulatus*) is a commonly consumed fruit worldwide and due to its sensorial and nutritional characteristics is extensively used as raw material in fruit juices industry (Mukhopadhyay et al., 2016). The actual consumers demand for high-quality and fresh-like fruit juices has been encouraging food industries to look for mild preservation techniques that minimize the negative impact of thermal treatments. Thermal processing is exceptionally effective on microbial reduction and control (Torlak, 2014), when conveniently applied. Nevertheless, high temperatures intensely modifies fruit juices quality, nutritional and sensorial characteristics (Sung, Song, Kim, Ryu, & Kang, 2014). Non-thermal processes are being applied and among them ultraviolet (UV) radiation appears as a promising food preservation process. In the short-wave range 200-280 nm of the UV spectrum (UV-C) the radiation has a germicidal effect, being lethal for the most types of microorganisms. It has been approved by U.S. Food Drug & Administration as a reliable alternative to thermal pasteurization of fresh fruit juice products (FDA, 2000; Koutchma, 2009). UV-C radiation allows to achieve a 5-$\log_{10}$ microbial reduction, mainly due to the photochemical reactions that are induced inside microorganisms and consequent injury of their DNA (Falguera, Pagán, Garza, Garvín, & Ibarz, 2011; Sastry, Datta, & Worobo, 2000); it is also considered economically attractive, easy to implement and environment-friendly (Guerrero-Beltrán & Barbosa-Cánovas, 2004; Miller, 2011). However, the efficacy of UV-C light depends on several factors, such as microorganisms species or intrinsic characteristics of juices (physical, optical and chemical) (Koutchma, 2009) and intensities or doses applied, which may explain the variability of published results. Usaga, Worobo, Moraru, and Padilla-Zakour (2015) reported more than 5 $\log_{10}$ cycles reduction of *Escherichia coli* in low turbidity apple juices. Tremarin, Brandão, and Silva (2017) attained similar results when applying UV-C radiation to *Alicyclobacillus acidoterrestris* spores in apple juices, after 8 minutes with an intensity of 13.44 W/m^2. However, Koutchma, Parisi, and Patazca (2007) only

achieved a reduction of 3.1 and 2.9 $\log_{10}$ cycles of *Escherichia coli* in orange and pineapple juices, respectively. Regarding intrinsic microflora of juices, lower reductions have been observed. Chia, Rosnah, Noranizan, and Wan Ramli (2012) reported for pineapple juice approximately 3 and 2 $\log_{10}$ cycles reductions of total mesophylls and yeasts and molds, respectively. Feng, Ghafoor, Seo, Yang, and Park (2013) reported 2.6, 1.5 and 1.0 $\log_{10}$ cycles reduction for coliforms, total aerobes and yeasts and molds, respectively, in watermelon juice. Moreover, several authors found that UV-C light exposure was very effective on juices' freshness, quality, nutritional and sensorial retention (Caminiti, Palgan, et al., 2012; Falguera et al., 2011; Gayán, Serrano, Monfort, Álvarez, & Condón, 2013; Shamsudin, Adzahan, Yee, & Mansor, 2014).

The main objective of this work was to assess the impact of UV-C radiation on the microbial decontamination and some quality attributes of melon juice. Juice was artificially inoculated with *Alicyclobacillus acidoterrestris* spores (spoilage indicators) and *Listeria innocua* (non-pathogenic surrogate of *L. monocytogenes*), selected as target microorganisms to determine the required UV-C light exposure time. Juice intrinsic microflora (total mesophylls and yeasts and molds), physicochemical characteristics (color, pH and soluble solids content), total phenolic compounds and antioxidant activity were also monitoring before and after treatments and during refrigerated storage.

2. Material and methods

2.1 Bacteria and spores cultures

A. acidoterrestris CCT 4384 spores' suspension of 10^7 CFU/mL with 10 days of incubation at 45 °C were obtained as described by Tremarin, Brandâo, and Silva (2017). *L. innocua* 2030c cultures of 10^7 CFU/mL at stationary phase were obtained as described by Miller, Gil, Brandão, Teixeira, and Silva (2009).

2.2 Juice samples preparation

Cantaloupe melons (*Cucumis melo* L. var. *reticulatus*) at commercial maturity stage were obtained at a local marketplace and stored overnight at 4°C. Melon juice was obtained using a domestic centrifuge (Centrifugal juicer Excel JE850, UK).

The UV transmittance (UVT) of the juice was measured in triplicate at 254 nm in a spectrophotometer (Specord® S600, Analytic Jena, Germany), using disposable polystyrene and acrylic cuvettes with 10 mm optical pathway. The average value of the melon juice transmittance was 10%.

Part of the juice (25.0 mL) was contaminated with 0.05 mL of *A. acidoterrestris* spores' suspension; another 25.0 mL of juice were inoculated with 0.05 mL of *L. innocua* culture.

2.3 UV-C radiation treatments

UV-C treatments were carried out in a camera (75 x 70 x 45 cm^3) with a bank of four germicidal UV lamps (TUV 15W/G15 T8, Philips, Holland) with peak emission at 254 nm designed by University of Algarve, Portugal. The methodology was the one described by Tremarin, et al. (2017). UV-C lamps were turned on for 30 minutes before treatments for stabilization. Juice samples (25.0 mL) were poured into Petri dishes with magnetic constant stirring (4 mm of juice thickness), which were placed on a supporting tray 30 cm below the lamps in a position that allowed an intensity of 13.44 W/m^2. Intensities of radiation were measured by placing a photo-radiometer (Delta OHMLP9021, Padova, Italy) at the same position of the samples.

For uncontaminated juices samples, UV-C radiation was applied for 5 and 20 minutes. For juices inoculated with *A. acidoterrestris* spores, samples of 1.0 mL were taken after 0, 3.0, 5.0, 8.0, 10.0, 15.0 and 20.0 min of exposure. For juices inoculated with *L. innocua*, samples of 1.0 mL were taken every 0.5 min till a maximum of 5 min of treatment. These experiments were conducted in different trials, each one with two sampling times maximum, to avoid significant variations of the juice sample thickness. Three replicates of each treatment were performed.

The power transmitted with UV radiation was used to estimate the thermal effect of processing. The juice temperature increment was calculated for the maximum exposure time, assuming that heat capacity of juice was the same as water. An increase of 0.87 °C was estimated, which was considered negligible for thermal effect.

2.4 Refrigerated storage

Untreated and UV-C treated juices for 20 min (40.0 mL) were placed into 100.0 mL sterile plastic containers and stored at refrigerated conditions (5 ± 2 °C) till a maximum of 13 days. Analyses were carried out at days 0 (before and after treatments), 1, 3, 6, 8, 10 and 13 and one plastic container was used for each sampling.

2.5 Microbiological analysis

Microbial analyses were carried out for untreated and UV-C treated juices.

For total mesophylls and yeasts and molds enumeration, juice decimal dilutions were carried out in buffered peptone water. Total mesophylls were assessed in duplicate, using Plate Count Agar (Lab M, Lancashire, UK). Samples were incubated at 37 °C, during 48 hours. Yeasts and molds were determined also in duplicate using Rose Bengal Agar (Lab M, Lancashire, UK). Samples were incubated at 25 °C for 60 h.

A. acidoterrestris spores were enumerated according to Silva, Gibbs, and Silva (2000), by spread plating the diluted samples onto *Bacillus acidoterrestris* agar (pH 4). The plates were incubated at 45 °C (Sanyo MIR-262) for 2–3 days. Microbial counts were performed in triplicate.

L. innocua was quantified in duplicate through decimal dilutions and using Palcam agar containing selective supplement (Merck, Darmstadt, Germany). Samples were incubated at 30°C for 3 days.

Enumerations were expressed as CFU per mL of juice.

2.6 Physicochemical determinations, total phenolics content and antioxidant activity

Determinations were carried out for untreated, UV-C treated juices and throughout refrigerated storage.

2.6.1 Color

Juice color coordinates (L*, a*, b*) were measured using a Minolta CR-400 colorimeter (Konica-Minolta, Osaka, Japan), calibrated before every measurement with a blank calibration plate. 25 mL of juice were placed in a petri dish whose cover had a hole through which the colorimeter beam reached the sample. Two readings of three different replicates were performed for each sample, always with the same light conditions. The brightness coordinate L* measures the whiteness value of a color from 0 (black) to 100 (white). The chromaticity value a* measures red when positive and green when negative, and the chromaticity coordinate b* measures yellow when positive and blue when negative. Total color difference (TCD) was calculated according to Eq. 1 and it is used to evaluate the color changes of untreated and treated samples (Alibas, 2009; Ihns, Diamante, Savage, & Vanhanen, 2011). Higher TCD values indicate more pronounced color deterioration.

$$TCD = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

In the above, the index “0” is related to untreated juice samples.

2.6.2 Soluble solids content and pH

Soluble solids content (SSC) of juices was directly measured using a Palette PR-32 digital refractometer (Atago, Tokyo, Japan). Results were expressed as °Brix. Juices pH was measured by using a pH meter (GLP 22, Crison Instruments, Spain). Measurements of SSC and pH were done in duplicate.

2.6.3 Total phenolics content

Extractions were performed by homogenizing 25.0 mL of juice in 50.0 mL of 100% methanol (Merck) with an Ultra-Turrax® homogenizer (Ika digital T25, IKA®-Werke GmbH

& Co. KG, Staufen, Germany). The mixture was centrifuged at 5000 × g for 10 min at 4 ºC. The remaining method is in accordance with the one described by Fundo et al. (2017). Briefly, the chromophore development reaction was based on oxidation of polyphenols via Folin-Ciocalteu reagent. Standard solutions were prepared using different concentrations of gallic acid. The reaction was performed by adding standard solution or juice sample to Folin-Ciocalteu's phenol reagent, Na_2C0_3 75 g L^{-1} and distilled water. After incubating for 1 h in the dark at room temperature, the absorbance was read in triplicate at 750 nm, using an UV/VIS spectrophotometer (Model 5625, ATI Unicam, UK). Total phenolics content of the samples (reported as µg gallic acid equivalent per mL of juice) was calculated by interpolation of the corresponding absorbance values in the calibration curve.

2.6.4 Antioxidant activity

Extractions were carried out as described for total phenolics determination and the ABTS assay was performed according to (Fundo et al. (2017)). Concisely, standard solutions were prepared using different ascorbic acid concentrations. The reaction was attained by adding standard solution or juice sample to the ABTS solution. After an incubation time of 6 min in the dark at room temperature, absorbance values were measured in triplicate at 734 nm. Total antioxidant activity (in g L^{-1} of ascorbic acid equivalent) was calculated by interpolation of the absorbance values in the calibration curve. Results were expressed as µg of ascorbic acid per mL of juice.

2.7 Statistical analysis

Differences between untreated and UV-C radiated juices and concerning all characteristics analysed were detected according one-way ANOVA. Normality and homoscedasticity of data was assessed using Shapiro-Wilk and Levene's tests, respectively. In situations when normality of data was not verified, the Kruskal-Wallis test was carried out alternatively to one-way ANOVA. Post-hoc tests were performed for

mean comparisons. Tukey's test was applied when normality of data was verified, and Mann-Whitney was the alternative non-parametric test used when data was not normally distributed. In all analyses carried out the significance level assumed was 5%. IBM SPSS Statistics 24 for Windows® (SPSS Inc., Chicago, USA) was used for data analyses.

3. Results and discussion

3.1 Impact of UV-C radiation

3.1.1 Microorganisms survival

The effectiveness of UV-C exposure was evaluated in terms of *L. innocua* and *A. acidoterrestris* spores' survival in juices. The initial juice contamination (N_0) was 10^5 CFU/mL for both *L. innocua* and *A. acidoterrestris* spores.

L. innocua was used as surrogate of the pathogenic *L. monocytogenes*, which is commonly used to assess thermal pasteurization efficacy (Miller, Gil, Brandão, Teixeira, & Silva, 2009). *A. acidoterrestris* is a fruit juice spoiler that produces undesirable off-favours, being a concern since its spores can survive to thermal treatments, even in acidic environments (Tremarin, et al., 2017).

The imposed intensity of UV-C radiation treatment for 5 and 20 minutes allowed two different doses applied, 4032 J/m^2 and 16128 J/m^2, respectively. The inactivation behaviours observed are presented in Figure 1, expressed as log of the ratio between microbial counts at a given time (N) and initial values. *L. innocua* was not detected after 5 minutes of radiation exposure, while 20 minutes were required for a total inactivation of *A. acidoterrestris* spores (3.7$\pm$0.3 $\log_{10}$ cycles reduction for *L. innocua* and 4.7$\pm$0.1 for *A. acidoterrestris* spores). These results showed that *A. acidoterrestris* spores were more resistant than *L. innocua*, requiring higher UV-C radiation doses for a safe

246 decontamination. Tremarin, et al. (2017) reported, for the same UV-C radiation intensity
247 of 13.44 W/m^2, a lower exposure time (8 minutes) to attain 5 log$_{10}$ cycles reduction of *A.*
248 *acidoterrestris* spores in apple juice (dose = 6451 J/m^2). This can be explained by the
249 differences of transmittance of the juices, which was significantly lower in the case of
250 melon due to juice turbidity.
251 In terms of juice intrinsic microflora (total mesophylls and yeasts and molds), the lowest
252 radiation dose did not affect the initial loads of these microorganisms, which were 5.6±0.3
253 and 4.6±0.2 log$_{10}$, respectively. For the highest dose applied, total mesophylls reduced
254 2.9±0.5 log$_{10}$ cycles and yeasts and molds reduced 1.4±0.2 log10 cycles.
255 Total mesophylls and yeasts and molds are a mix of microorganisms, which may have
256 different resistance to UV-C radiation. This may explain the lower reductions observed,
257 when compared to the ones obtained for *L. innocua* and *A. acidoterrestris* spores (Habibi
258 Najafi & Haddad Khodaparast, 2009). Even, between strains of the same species,
259 different results may be attained (Gabriel, 2012). Chang et al. (1985) and Sastry et al.
260 (2000) referred that, generally, gram-negative bacteria are the most sensitive
261 microorganisms followed by gram-positive bacteria, viruses, fungi, spores, and cysts.
262 Fruit matrix, microorganisms and doses of radiation applied are influencing factors in
263 decontamination. Regarding orange juice, 36090 J/L resulted in 2.8, 0.3 and 5.7 log$_{10}$
264 cycles reductions in aerobic plate counts, yeasts and molds and *E.coli*, respectively (Pala
265 & Toklucu, 2013). Studies with apple juice revealed reductions of 4.8 log$_{10}$ cycles of *E.*
266 *coli* at 1.52 W/m^2 (Koutchma, et al., 2007) and of 2.1 log$_{10}$ cycles of *A. acidoterrestris*
267 spores at 5390 J/m^2 (Baysal, Molva, & Ünlütürk, 2013). In grape juice, *Saccharomyces*
268 *cerevisiae* was reduced by 3.39 ± 0.04 log$_{10}$ cycles with UV dose of 655.0 J/m^2 (Kaya,
269 Yıldız, & Ünlütürk, 2015). Published results are expressed as radiation intensity or dose
270 applied, which makes comparison hard. Quite often other experimental conditions used
271 are not clearly presented and conversion of units for uniformization of radiation
272 conditions is not possible.
273

3.1.2 Physicochemical characteristics

The effects of 5 and 20 minutes of UV-C radiation exposure were evaluated in terms of color parameters, soluble solids content and pH (Table 1). Total color difference was calculated in order to have a global perception of juice color alterations. Accordingly to Drlange (1994), the alterations observed in melon juice treated samples can be classified as very distinct, since TCD values are between 3 and 6. However, no significant differences were attained between UV-C exposure times. Although some authors pointed out a significant impact of radiation on juices color individual coordinates (L^*, a^*, b^*), the significant difference obtained for melon juice was only for L^* when the treatment was applied for 20 minutes. This can be related to the high concentration of color pigments present in this juice (Fundo et al., 2017), which may provide a masking effect on color differences (Lee & Coates, 1999; Taze, Unluturk, Buzrul, & Alpas, 2015).

The radiation exposure did not affect the soluble solids content of melon juice. These results are in accordance with reported data (Cava & Sgroppo, 2015; Noci et al., 2008; Unluturk & Atilgan, 2015).

In terms of pH, significant differences were observed between untreated and treated samples and also between different exposure times, with a slight pH increase as treatment time increases. This may be explained by the degradation of some acid compounds, such as vitamin C, present in significant quantities in melon juice (more than 100mg/100g of juice; Fundo et al., 2017). However, pH alterations are not commonly referred (Caminiti, Noci, Morgan, Cronin, & Lyng, 2012; Cava & Sgroppo, 2015; Noci et al., 2008; Unluturk & Atilgan, 2015),

3.1.3 Total phenolics content and antioxidant activity

Total phenolics content and antioxidant activity of melon juice were not significantly affected by UV-C radiation (Table 1). However, Ochoa-Velasco and Beltrán (2013) attained a reduction of 11.6% on phenolic compounds and 37% on antioxidant activity in pitaya juice treated at 1026 J/m^2. Ochoa-Velasco and Beltrán (2013) and Sew, Ghazali,

Martín-Belloso and Noranizan (2014) also observed in pineapple juice a decrease of total phenolics content when UV-C doses increased from 56.1 to 112.3 J/m^2, mainly due to the oxidation promoted by free radicals produced during juices light exposure. Those free radicals may also prompt stress responses such as accumulation of phytoalexins (Alothman, Bhat, & Karim, 2009) or increase antioxidants extractability (Bhat, Ameran, Voon, Karim, & Tze, 2011), allowing detection of higher antioxidant activity in radiated fruit products.

3.2 Refrigerated storage

3.2.1 Microorganisms survival

The highest dose of UV-C radiation (20 minutes of melon juice exposure) was chosen as the treatment to apply before juice storage at refrigerated conditions. This treatment allowed a pre-decontamination of juices in terms of *L. innocua* and *A. acidoterrestris* spores. Total mesophylls and yeasts and molds were the microorganisms monitored during the storage period (Figure 2). Due to UV-C impact, and before storage, the loads of these microorganisms were 2.7±0.2 and 3.1±0.3 $\log_{10}$ cycles for total mesophylls and yeasts and molds, respectively.

Total mesophylls loads increased during the storage and no significant differences were observed between untreated and treated samples (Figure 2a). Since total mesophylls are a group of microorganisms that include a remarkable number of species, some of them may have repair mechanisms in response to damages caused by the UV-C light exposure (Keyser, Műller, Cilliers, Nel, & Gouws, 2008). However, for yeasts and molds, significantly different behaviours were observed for untreated and UV-C radiated juices throughout storage (Figure 2b). In samples that had not suffer radiation, yeasts and molds grew, while in treated ones the initial load was maintained constant till the end of storage, with no capacity to recover.

330 3.2.2 Physicochemical characteristics, total phenolics content and antioxidant activity

331 Color alterations of juice were evaluated throughout refrigerated storage (Figure 3a). UV-
332 C exposure had a positive impact on color maintenance since, at the end of the storage
333 period, TCD of treated samples was significantly lower than untreated ones. Small color
334 alterations were observed for treated samples, while for untreated ones the differences
335 were distinct. Color changes are a result of chemical and biochemical reactions
336 (formation of brown pigments or carotenoids fading) that may happen during the storage
337 (Wibowo et al., 2015). In UV-C treated samples, these reactions may have occurred in
338 less extension. In terms of pH (Figure 3b), UV-C treated juices had a significant pH
339 increase, from day 0 to day 13, while in untreated samples pH did not vary. No significant
340 differences were observed between soluble solids content of untreated and treated
341 samples during storage (results not shown). Total phenolics content were not different in
342 untreated and treated juices (Table 2). At the end of storage the values did not vary
343 significantly. However, the antioxidant activity of untreated juices decreased significantly
344 after 13 days of storage (around 58% of decay), while for UV-C radiated samples the
345 antioxidant activity was retained. Some works refer a decrease of antioxidant activity of
346 juices during storage, due to vitamins and pigments decay (Cava & Sgroppo, 2015;
347 Ochoa-Velasco & Beltrán, 2013). Some stress responses during storage may induce the
348 synthesis of some bioactive compounds, leading to the maintenance of overall
349 antioxidants activity.

350

351

352 **4. Conclusions**

353

354 UV-C radiation can be considered as a promising non-thermal alternative for microbial
355 decontamination of melon juice, since 5 and 20 minutes of exposure allowed attaining a
356 reduction of 3.7±0.3 $\log_{10}$ cycles for *L. innocua* and 4.7±0.1 for *A. acidoterrestris* spores.
357 Concerning juice intrinsic microflora inactivation assessed in terms of total mesophylls

and yeasts and molds, UV-C radiation was less efficient. Nevertheless, during the refrigerated storage for 13 days, yeasts and molds in UV-C treated juices did not grow. Additionally, color and antioxidant activity were better retained when juices suffered UV-C radiation before storage.

Although the efficacy of UV-C light exposure depends on intrinsic characteristics of juices and exposure doses, the attained results revealed that this non-thermal technology can be considered as a suitable alternative to traditional pasteurization. However, the radiation apparatus used in this experience (overhead exposure) works in batch and therefore it is not feasible for large volumes of juice. To overcome this problem, continuous systems are being developed for industrial scale up of this technology, such as thin film or coiled tube reactors.

Conflict of interest

There are no conflicts of interest regarding this paper.

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Figure captions

Figure 1. Inactivation of *L. innocua* (●) and *A. acidoterrestris* spores (○) in melon juice under UV-C radiation exposure. The values are mean ± margin of confidence interval at 95%.

Figure 2. Total mesophylls (a) and yeasts and molds (b) growth during storage of untreated (■) and of UV-C treated melon juices (□). The values are mean ± margin of confidence interval at 95%.

Figure 3. Total color difference (a) and pH (b) during storage of untreated (■) and of UV-C treated melon juices (□). The values are mean ± margin of confidence interval at 95%.