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Evolution during refrigerated storage of bioactive compounds and quality characteristics of grapefruit [*Citrus paradisi* (Macf.)] juice treated with UV-C light

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Highlights

►The ascorbic acid content and the antioxidant capacity decreased after the treatment. ►The treatments had not effect in flavonoids, phenol and organic acids contents. ►The colour of grapefruit juice was unaffected after UV-C treatment. ►The UV-C treatment delayed microbial growth during refrigerated storage.

Abstract

The effect of the UV-C light (doses: 0.0 to 3.94 J/cm²) on the main bioactive compounds of grapefruit juice and their stability were evaluated throughout 30 and 16 days of storage at 4 and 10 °C respectively. Organic acids (citric, malic, ascorbic and tartaric) and flavonoids (naringin, hesperidin and neohesperidin) were quantified by HPLC, whereas total phenols and the antioxidant capacity were determined by spectrophotometric methods. The UV-C treatments caused a significant decrease (15% to 30%) in ascorbic acid and antioxidant capacity (10% to 27%), which was related to the applied dose. However, no changes ($p>0.05$) in others organics acids, individual flavonoids, total

phenols, pH, °Brix, colour and titratable acidity were observed after UV-C treatment. During the storage at both temperatures, a decrease in the neohesperidin levels (43% - 53%) was detected whereas the others parameters analyzed did not show changes ($p>0.05$). The microbiological quality of grapefruit juices treated with 3.94 J/cm² was maintained for 15 and 10 days at 4 and 10º C respectively.

Keywords: non-thermal technology; organic acids; naringin; antioxidant capacity; microbiological quality;

1. Introduction

The grapefruit [*Citrus paradisi* (Macf.)] juices are produced by industries all over the world due to the preference of the consumer based on its taste. Furthermore, they have high nutritional values and health-promoting compounds, being the ascorbic acid one of the most important. Ascorbic acid is the main compound with vitamin C activity and is a natural antioxidant that may inhibit the development of major oxidative human reactions. This compound, together with citric, malic and tartaric acid contribute to flavour attributes and are used as ‘‘fingerprints” to detect the quality of the juice (Cen, Bao, He, & Sun, 2007). Other bioactive compounds present in the grapefruit juice are the flavonoids, which are associated with biological properties, including antioxidant activity, drug interactions (de Castro, Mertens-Talcott, Derendorf, & Butterweck, 2007), anti-inflammatory and anti-tumor effects (Fujita et al., 2008; Kim, Lee, Lee, Park, Kim, & Moon, 2008). The naringin is the main flavonoid in grapefruit juice and it is responsible for its bitter taste. Other neohesperidosides are present in fewer amounts, such as neohesperidin, hesperidin, poncirin and neoeriocitrin (Igual, García-Martínez, Camacho & Martínez-Navarrete, 2011). Currently, there is a strong demand for technologies ensuring the stability of the bioactive compounds in foods (Lopez-Rubio, Gavara & Lagaron, 2006).

Traditionally, fruit juices have been pasteurized by heat treatment in order to prolong their shelf life. However, this treatment may cause irreversible losses of nutritional quality and antioxidant activity in the juice, thereby adversely affecting their properties health-related. On the other hand, non-thermal technologies for food processing are receiving great attention due to the ability to improve the quality and safety of foods. The UV-C light was suggested as one of the non-thermal technologies capable of ensure the microbial safety of fruit juices retaining their nutritional properties (Falguera, Garza, Pagán, Garvín & Ibarz, 2013; Uysal Pala & Kirca Toklucu, 2011). The scientific criteria accepted for pasteurization of juices through a non-thermal technology UV-C is a 5 log reduction of the microorganism target (NACMCF, 2006). Moreover, the process requires very little energy compared to thermal pasteurization, also remove any traces of pesticides and it is not harmful for workers and the environment (Guerrero-Beltran & Barbosa- Canovas, 2005; Koutchma, Forney & Moraru, 2009). The radiant energy emitted at 254nm (112.8 Kcal/Einstein) could affect the O-H, C-C, C-H, C-N, H-N and S-S bonds if it is absorbed. Additionally, this energy induces the crosslinking of neighbouring pyrimidine nucleoside bases in the same DNA strand, blocking DNA transcription and replication and eventually causing the cell death (Guerrero-Beltran & Barbosa-Canovas, 2005).

Although, the effect of UV-C light on the main quality characteristics have been reported in juices of orange (Tran & Farid, 2004), apple (Noci et al., 2008), pomegranate (Uysal Pala & Kirca Toklucu, 2011), starfruit (Bhat, Ameran, Ching Voon, Karim & Min Tze, 2011) and grape (Falguera et al., 2013), no works has been carried out to study the effects of the UV-C light on the organic acids, flavonoid contents and their changes during refrigerated storage of grapefruit juice. The aim of this work was to evaluate the effects of UV-C light on the levels of citric, ascorbic, malic and tartaric acids, as well as naringin, neohesperidin and hesperidin of grapefruit juice. Moreover, the evolution of these compounds during storage at 4 and 10 ºC were studied. Additionally, microbial growth, pH, °Brix, titratable acidity, colour changes, total phenols, and antioxidant capacity were analyzed.

2. Materials and Methods

2.1. Preparation of Juice

The grapefruits [*Citrus paradisi (*Macf.*)*] cv 'Duncan', with uniform coloration of skin, free of cuts, similar weight and size, ratio=5.5, were provided by the Estación Experimental INTA Bella Vista (Corrientes, Argentina, -28 ° 30` 52.43`` N, -59 ° 1` 47.94`` S). The fruits were washed with tap water, sanitized (HClO, 200ppm/5min), rinsed and squeezed with a domestic extractor. The juice was filtered through a sieve (mesh aperture of 3-4 mm) before the treatments.

2.2. UV-C treatments and storage conditions

The UV-C treatments were carried out in a chamber size of 150 cm x 100 cm x 60 cm stainless steel construction, equipped with three UV-C germicidal lamps (254nm, UV, TUV 36W/G 36 T8 Phillips), mercury low pressure (Figure 1). The UV radiation intensity average reached to the sample surface was quantified by chemical actinometry using an iodide/iodate solution in an area equivalent to the treatment surface (Rahn, 1997). The incident photons were calculated by assuming that, being the mixture optically opaque below 290 nm, all of the incident photons were absorbed by the solution. In each experience, a volume of 200 mL of fresh grapefruit juice was placed in a container Pyrex (27 cm x 11 cm) forming a film thickness of 5-7 mm under magnetic stirring (Precytec modelo AE-29, Argentina). The excess of heat generated inside the chamber was dissipated with a fan, controlling the temperature never exceeded 25 ± 1 °C. The distance between the surface of grapefruit juice and the lamps was 17 cm. Doses of 0.0, 1.83, 2.84 and 3.94 J/cm^2 were applied to grapefruit juices during the experiences. Previously, we determined that higher doses than 1.83 J/cm^2 were effective to decrease more than 5 cycles log cfu/mL of *E. coli* ATCC 25922 (data not published), close to those suggested to pasteurize orange juice by Oteiza et al. (2010).

After the irradiation process, the samples were placed in sanitized conical containers of polypropylene (50 mL) with screw cap and stored as follows: at 4 ºC three tubes were taken randomly without replacement for each dose at days 0, 5, 10, 15, 20, 25 and 30. At 10 ºC three tubes were taken randomly without replacement for each dose at days 0, 4, 8, 12 and 16. The whole experience was performed at least 2 times.

2.3. Content of organic acids

The determination of tartaric, malic, ascorbic and citric acid was carried out by the method of Scherer, Rybka, Ballus, Meinhart, Teixeira Filho and Teixeira Godoy (2012). The organic acids contents were quantified by high performance liquid chromatography (Shimadzu LC- 10A, Tokyo, Japan) coupled with Hypersil ODS C_{18} (250mm x 4.6 mm, 5 µm particle size, Thermo Scientific, Whatman, MA, USA) column and the UV-visible diode array detector (Shimadzu, SPD-M20A, Tokyo, Japan) fixed at 210 nm for tartaric, malic and citric acid and 254 nm for ascorbic acid. The mobile phase was 0.01 mol/L KH_2PO_4 buffer solution (pH = 2.60 adjusted with o-phosphoric acid), with a flow rate of 1.0 mL/min. The samples were prepared with 5 mL of grapefruit juice mixed with equal parts of mobile phase and filtered through a 0.45 µm nylon membrane previously to injection of 20 µL. The results were expressed as mg/100 mL grapefruit juice based on the standard curve prepared with patterns of each acid in a range of 20 - 40 mg/100 mL (Sigma –Aldrich, St. Louis, MO, USA).

2.4. Separation and quantification of flavonoids

Five mL of grapefruit juice and 5.0 mL of a solution of ammonium oxalate 0.025 mol/L were mixed in a tube, 5 mL of dimethylformamide was added, stirred and finally H_2O was added to fill up 25 mL. Subsequently the mixture was heated for 10 minutes at 90 °C, and an aliquot filtered through a membrane filter after cooling. Twenty µL of this solution was injected into the high performance

liquid chromatograph (Shimadzu LC-10A, Tokyo, Japan) coupled with Hypersil ODS C_{18} (250mm x 4.6 mm, 5 µm particle size, Thermo Scientific, Whatman, MA, USA) column and the UV-visible-diode array (Shimadzu, SPD-M20A, Tokyo, Japan) detector fixed at 280 nm for naringin, hesperidin and neohesperidin. The mobile phase of acetonitrile: water: acetic acid (20:79.5:0.5) with a flow rate of 1.2 mL/ min. The results were expressed as mg/100 mL of grapefruit juice using standard curves prepared with patterns of each flavonoid (Sigma –Aldrich, St. Louis, MO, USA) in a solution of dimethylformamide: 0.01 M acetic acid (20:80).

2.5. Main physicochemical parameters

The grapefruit juice UV absorptivity was determined at 254 nm (Metrolab 1700 UV-VIS) according to Oteiza et al. (2010) and turbidity with a Triton Turbidimeter (Parsen Company, Buenos Aires, Argentina). The soluble solids (°Brix) and pH were measured at 25 °C using a refractometer (Model Ref 107 HandHeld, China) and a pH-meter (Metrohm meter pH-/ion, Switzerland). The titratable acidity was determined potentiometrically with 0.1 N NaOH and expressed as g of citric acid/100 mL of grapefruit juice.

2.6. Colour

The colour of the fresh and treated grapefruit juice was measured with a colorimeter Minolta CR-400 Chroma Meter (Konica Minolta Sensing, Inc., Osaka, Japan). The L*, a*, b* parameters were measured and ΔE* was calculated by $((L^*_o-L^*)^2 + (a^*_o-a^*)^2 + (b^*_o-b^*)^2)^{1/2}$, where L^*_o, a^*_o and b^*_o were measured for grapefruit juice control at the beginning of the experiment.

2.7. Total phenols and antioxidant capacity

The total phenolic content was determined with the Folin-Ciocalteu reagent (Singleton & Rossi, 1965) using 50 µL of grapefruit juice. The results were expressed as mg of gallic acid equivalents

(GAE) per 100 mL of grapefruit juice. The free radical scavenging activity of grapefruit juice was measured according to the DPPH• method suggested by Kelebek (2010).

2.8. Microbiological analyses

Total aerobic count was determined by using serial dilutions on plate count agar (Britania, Argentina) with a pour plate method. Serial dilutions in a range of 10^{-1} to 10^{-6} of treated and control grapefruit juices were performed with sterile 0.1% peptone water. The duplicate plates were incubated at 35 $\pm$ 2 °C for 48 hours. The count of the total yeasts and moulds with the same dilutions was carried out on yeast extract, potato dextrose agar (Britania, Argentina) at 25 °C during 5 days using the pour plate method. Results were expressed as log colony-forming units per mL (log cfu/mL) (AOAC 2000). The growth rate constant (μ) was calculated using $N_2 = N_1 \exp [\mu (t_2 - t_1)]$ where N_1, N_2 are the cfu/mL at times t_1, t_2 (Painter and Loveless, 1981).

2.9. Statistical analysis

The experiments were performed in duplicate for each condition. The result of each determination was expressed as the mean of 3 determinations. Significant differences were evaluated by ANOVA and Duncan test ($p < 0.05$) using the Info-Stat Statistical Software (Cordoba-Argentina, 2009). The Pearson correlation coefficient (R) was used ($p<0.01$) to explain the relationship between the different compounds quantified and antioxidant capacity of the grapefruit juice.

3. Results

3.1. Organic acids

The predominant acid in grapefruit juice is the citric acid, whose values were in the range of 1584 $\pm$ 20 mg/100 mL to 1759 $\pm$ 2 mg/100 mL. The malic acid content was between 37.4 $\pm$ 0.2 mg/100 mL and 42.7 $\pm$ 1.2 mg/100 mL, whereas the tartaric acid content was between 12.0 $\pm$ 0.4 mg/100 mL and

175 48.9 ± 3.2 mg/100 mL (Table 1 and 2). Similar contents were reported for others varieties of
176 grapefruits (Igual, García-Martínez, Camacho & Martínez-Navarrete, 2010; Uckoo, Jayaprakasha,
177 Somerville, Balasubramaniam, Pinarte & Patil 2013).
178 After UV-C treatment, citric and malic acid levels were unchanged ($p<0.05$) as was observed in
179 orange juice treated in a range of 12.03 to 48.12 kJ/L (Uysal Pala & Kirka Toklucu, 2013). The citric
180 acid levels of control and grapefruit juice treated with 1.83 J/cm^2 showed losses of 5-7 % ($p>0.05$) at
181 the end of storage at 4 °C, whereas the juices treated with doses higher than 2.84 J/cm^2 remained
182 unchanged (Table 1). On the other hand, the malic acid content decreased between 14% and 20% for
183 control and UV-C treated grapefruit juice (1.83 and 2.84 J/cm^2) at 30 days. Meanwhile, in grapefruit
184 juice treated with 3.94 J/cm^2 losses lower than 4% were detected (Table 1). The tartaric acid content
185 was unchanged during storage at 4 °C.
186 The initial ascorbic acid content in grapefruit juice cv. 'Duncan' was between 41.0 ± 0.6 mg/100 mL
187 and 56.9 ± 0.6 mg/100 mL, in the order of those reported by Uckoo et al. (2013) and Igual et al.
188 (2010). The ascorbic acid content was significantly reduced by UV-C treatment ($p<0.05$), being the
189 losses between 12-17%, 20-29% and 25-35% after the application of 1.83, 2.84, 3.94 J/cm^2
190 respectively (Table 1 and 2). However, in orange juices treated under continuous system, the
191 ascorbic acid decreased more than 9 % (Uysal Pala & Kirka Toklucu 2013; Tran & Farid 2004),
192 whereas in grape juice it was more noticeable (30%) (Falguera et al., 2013). Tikekar,
193 Anantheswaran, Elias & LaBorde (2011) suggested that the mechanism for UV-induced ascorbic
194 acid degradation in juices is similar to the general mechanism for metal-catalyzed oxidation.
195 Moreover, the decrease in the ascorbic acid content could be related to the coincidence between its
196 absorption maximum and the peak of emission of UV-C lamps. The ascorbic acid content in
197 untreated grapefruit juice remained without changes ($p>0.05$) during the storage at 4 ºC. Meanwhile,
198 all samples treated with UV-C did not show statistically significant changes ($p>0.05$) during the first
199 20 days of storage at 4ºC, then, a gradual decrease was observed, with losses between a 9-14 % at

200 day 30 ($p < 0.05$) (Table 1). At 10 ºC the organic acids levels of treated and control juices remain
201 unchanged during the 16 days of storage (Table 2).
202
203 **3.2. Flavonoids**
204 Flavanones constitute the 98 % of the total flavonoids present in grapefruits being well known for
205 several health promoting properties. The naringin was the main flavonoid found in grapefruit juice
206 and values were between 17.5 $\pm$ 0.6 mg/100 mL and 27.8 $\pm$ 1.7 mg/100 mL. The neohesperidin
207 values were between 1.3 $\pm$ 0.3 mg/100 mL and 2.5 $\pm$ 0.6 mg/100 mL, whereas the hesperidin was not
208 detected (Tables 1 and 2). These values were close to those reported by Uckoo et al. (2013) and Igual
209 et al. (2011) in other varieties of grapefruits. The naringin and neohesperidin levels in grapefruit
210 juice remained unchanged ($p>0.05$) after UV-C application (Table 1 and 2).
211 On the other hand, the naringin content of the treated and control grapefruit juices was unchanged
212 and showed similar behaviour during storage at 4 and 10ºC ($p>0.05$). However, the neohesperidin
213 level showed losses of 43-58 % after 15 days of storage in all grapefruit juices (Table 1 and 2**).**
214 According to our knowledge, there are no reports about the effects of UV-C treatment on individual
215 flavonoids of grapefruit juice; however, there are several reports concerning the effects of UV-C
216 radiation on total flavonoids of other juices. In pineapple juice treated with 7.5 mJ/cm^2, the total
217 flavonoids were unchanged (Goh, Noranizan, Leong, Sew & Sobhi, 2012), however in starfruit juice
218 increases were found after irradiation with doses of 2.158 J/m^2 (Bhat et al., 2011).
219
220 **3.3. Main physicochemical parameters**
221 The UV absorptivity and turbidity of grapefruit juice were of 49.47 cm^{-1} and 2500 NTU respectively.
222 The value of UV absorption coefficient was close to those reported for orange and guava juice and
223 the turbidity was between the values of apple juice (900 NTU) and orange juice (3759 NTU)
224 (Koutchma et al. 2009). The values of pH, ° Brix, and titratable acidity are presented in Table 3 and

4 for control and treated grapefruit juice. After the UV-C application and during refrigerated storage at both temperatures, there were no significant changes in those parameters ($p>0.05$) as was observed in other UV-C treated juices (Falguera et al., 2013; Bhat et al., 2011; Caminiti, et al., 2011).

3.4. Colour

The colour is one most important criterion for consumer preference and it is measured as a parameter of juice quality. Immediately after UV-C treatment were detected differences lesser than 1.5 for ΔE* (Table 3 and 4) close to those reported by Noci et al. (2008) in apple juice UV-C treated. These differences are `slightly noticeable´ according to the classification used by Caminiti, Noci, Morgan, Cronin & Lyng (2012). A gradual trend of increased in ΔE* were observed during storage at both temperatures, mainly due to increases of L*, however these values did not exceed 2.5. Browning was not detected in any juice during storage.

3.5. Total phenols and antioxidant capacity

The total phenols content were in the range of 68.9 $\pm$ 2.6 mg /100 mL to 86.5 $\pm$ 3.7 mg /100 mL, close to those reported for other varieties of grapefruit juice (Igual et al., 2010). The total phenols content after UV-C treatment did not show statistically significant changes ($p>0.05$), as was observed in orange (Uysal Pala & Kirka Toklucu, 2012), although in others fruit juices the behaviour was unevenly (Falguera et al. 2013; Noci et al., 2008). Throughout the storage period, statistically significant changes ($p< 0.05$) were observed in the total phenol contents at both temperatures, which resulted in a percentage loss of the 14 % for control grapefruit juices and between 11 % and 20 % for treated UV-C samples at the end of storage (Table 3 and 4).

The antioxidant capacity was determined by the free radical-scavenging DPPH• reactive and values expressed as EC50%, being the lowest values related with a highest antioxidant activity of the compounds. The antioxidant capacity in the fresh grapefruit juice was $0.0025 \pm 7.1\text{x}10^{-5}$ mL/mg,

250 which was higher than those determined in other grapefruit juices (Kelebek, 2010). The antioxidant
251 capacity showed losses of 10 %, 22.5 % and 27% after UV-C treatment with 1.83, 2.84 and 3.94
252 J/cm^2 respectively. These results are in discrepancy with those reported for orange and apple juices
253 UV-C treated in continuo systems (Uysal Pala & Kirka Toklucu, 2012, 2011; Noci et al., 2008).
254 During refrigerated storage the antioxidant capacity values of control and UV-C treated grapefruit
255 juice remained without changes ($p>0.05$) (Table 3 and 4).
256
257 **3.6. Microbial analyses**
258 The grapefruit juices recently squeezed had low loads of total aerobic and yeast and moulds and they
259 were very close to the limit of detection (<1.0 log cfu/mL). During storage at 4 ºC, the control juices
260 showed a rapid increase in total aerobic and yeast and moulds loads (2.02 and 2.61 log cfu/mL,
261 respectively) after 10 days and increased to 5.30 log and 5.15 log at day 15, remaining unchanged
262 until the end of storage (Figure 2). However, the juices treated with 1.83 J/cm^2 showed an increase in
263 the total aerobic and yeast and moulds counts of 1.55 and 2.12 respectively after 10 days, whereas in
264 grapefruit juice treated with 2.84 and 3.94 J/cm^2 the aerobic microbial growth were not observed. At
265 day 15, numbers of total aerobic and yeast and moulds showed rapid growth in all grapefruit juices,
266 being at the end of the storage the difference between the control and UV-C treated juices lesser than
267 1 log cfu/mL (Figure 2). During storage at 10 ºC, the control juice had counts of 1.32 and 1.16 in
268 total aerobic and yeast and moulds at day 8, after that the counts increased rapidly (3.38 and 3.08),
269 remaining unchanged until 16 days of storage. A similar behaviour was observed in grapefruit juice
270 treated with 1.83 J/cm^2 with differences of less than 1 log cfu/mL (Figure 3). However, total aerobic
271 and yeast and moulds count of treated grapefruit juice with 2.84 and 3.94 J/cm^2 were <2 log cfu/mL
272 throughout 16 days storage at 10 ºC. At both storage temperatures the UV-C treatments were able to
273 retard microbial growth in the range of 10 to 15 days and the lowest microbial load was detected

with the highest doses applied. This was in agreement with the results obtained by Uysal Pala & Kirka Toklucu (2013) and Tran & Farid (2004) in UV-C treated orange juice.

4. Discussion

The individual flavonoids and total phenols, as well as citric, malic and tartaric acid contents did not show changes after the UV-C treatment, whereas the ascorbic acid and antioxidant capacity contents decreased significantly ($p<0.05$), being more noticeable with higher UV-C doses.

In order to explain the relationships between the different compounds quantified and antioxidant capacity of the grapefruit juice, the Pearson correlation coefficient (R) and p-values were used. The naringin content correlated highly ($R=0.95$, $p=0.001$) with total phenols, which may be related to the polyphenolic structure of naringin. However, the naringin content showed not significant correlation with antioxidant capacity ($p=0.220$). Likewise, Amic, Davidovic-Amic, Beslo & Trinajstic (2003) found that flavonoids without 3-OH and 3`,4` di-OH had low antioxidant capacity measured through radical scavenger DPPH •. Moreover, the antioxidant capacity measured through DPPH• correlated highly ($p=0.004$) with ascorbic acid content, compound that was reported as the main antioxidant in many fruit of *Citrus* genus (Del Caro et al., 2004).

During the storage at both temperatures, ascorbic acid content and antioxidant capacity in UV-C treated and untreated grapefruit juice remained unchanged ($p<0.05$), which could be related to the insignificant headspace of the packaging and the negligible O_2-permeability of the polypropylene tube. The other organic acids studied did not show changes in grapefruit juice treated with doses higher than 2.84 J/cm^2, probably due to the low load of spoilage microorganism (Chia, Rosnah, Noranizan & Ramli, 2012). The neohesperidin content in grapefruit juice gradually decreased during storage, with notable losses after 15 days. The naringin levels, total phenols, pH, colour, ºBrix and titratable acidity remained unchanged ($p>0.05$).

The shelf-life of fresh citrus fruit juice is limited during storage by reduction in organoleptic quality and development of microorganisms (Tran & Farid, 2004). In our work, may be obtained two conclusions by relating the UV-C dose applied and microbiological evolution during storage conditions. First, the total aerobic and yeast and moulds counts were lower when increasingly higher doses were applied, which could be related to the higher damage at the DNA level (Tran & Farid, 2004). Second, in all treated grapefruit juices the microorganism growth was delayed for a longer time compared with untreated ones. Jungfer, Schwartz & Obst (2007) reported that the delay in microbial growth is proportional to the damage received during the treatments and the type of microorganism. Supporting that, at 4 °C the growth rate constants for total aerobic were of 0.81, 0.69, 0.72 and 0.62 day^{-1} and for yeast and moulds were of 0.79, 0.76, 0.64 and 0.59 day^{-1} for doses of 0.0, 1.83, 2.84 and 3.94 J/cm^2 respectively. During storage at 10 °C the growth rate constants for total aerobic were of 0.49, 0.48, 0.28 and 0.10 day^{-1} and for yeast and moulds were of 0.44, 0.36, 0.31 and 0.13 $day^{-1,}$ when treatments of 0.0, 1.83, 2.84 and 3.94 J/cm^2 were applied. At both temperatures of storage, the samples treated with UV-C showed a decrease in the growth rate constant for total aerobic and yeast and moulds compared with untreated, and the decrease were related with the intensity of applied doses. Also it should be noted that, the presence of filamentous micro-structures in juice-air interface of samples stored was the main alteration signs and they are related to the growth of moulds and was observed in treated juices after 15 and 10 days of storage at 4 and 10ºC respectively. Yeasts and moulds have more resistance than other bacteria probably due to DNA structure and the chemical composition of the cell wall and its thickness (Tran & Farid, 2004). Meanwhile, Uysal Pala & Kirka Toklucu (2013) found similar behaviour at 4 and 10 °C on microbial growth, reported that differentiations of physicochemical characteristics of fruit juices including pH, soluble solids and phenolic compounds may have had significant effects on microbial growth during storage in addition to the effects of storage temperature. Also, Ahmed, Chandan, Mukund, Sumeet & Chidambaram (2014) reported that the orange juices with more citric acid content showed lower

323 microbial load. This was in agreement with Bizri & Wahen (1994) who found differences as high as
324 2 logarithmic cycles in the total aerobic counts in tomato juice with different pH values (less than
325 0.4).
326
327 **Conclusion**
328 The UV-C treatments decreased ascorbic acid and antioxidant capacity of grapefruit juice and the
329 effect was more noticeable when higher doses were applied. However, the naringin, neohesperidin,
330 citric, malic, tartaric acid as well as, pH, ºBrix, titratable acidity, colour and total phenols were not
331 affected.
332 During the refrigerated storage, the treatments with UV-C enhanced the shelf life of juices for 15 and
333 10 days at 4 and 10 ºC respectively, due to the microbiological control achieved. The treatments
334 were not effective to prevent loss of neohesperidin and total phenols during storage at both
335 temperatures, while organic acids had a lower degradation in treated grapefruit juice.
336 Also, the naringin and ascorbic acid contents, as well as antioxidant capacity, pH, ºBrix, titratable
337 acidity and colour showed similar evolution in treated and control grapefruit juice for both storage
338 temperatures. Then, the UV-C treatments could be suggested as a method for preservation of
339 grapefruit juice, if they are accepted sensorially by the consumers.
340
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Table 1. Organics acids and individual flavonoids content of untreated and UV-C treated grapefruit juices during 30 days refrigerated storage at 4 °C.

			Organic Acids (mg/ 100 mL)				Flavonoids (mg/ 100 mL)	
Treatment	**T (°C)**	**Days**	**Citric**	**Malic**	**Tartaric**	**Ascorbic**	**Naringin**	**Neohesperidin**
untreated	4	0	1759 A-a	37.4 A-a	12.7 A-abc	41.0 A-a	17.5 A-bc	1.51 A-a
		5	1725 AB-ab	37.4 A-a	12.1 A-ab	39.9 A-ab	18.9 A-a	1.12 A-bc
		10	1704 A-ab	37.2 A-a	12.5 A-abc	39.1 A-ab	16.6 A-c	1.25 A-ab
		15	1695 AB-ab	37.2 A-a	11.6 AB-a	36.5 A-b	17.0 A-bc	0.83 A-c
		20	1704 A-ab	33.8 A-b	12.8 AB-abc	38.5 A-ab	17.4 A-bc	0.72 A-c
		25	1682 A-ab	31.8 A-bc	13.7 A-c	38. 0 A-ab	17.8 A-abc	0.86 A-c
		30	1667 AB-b	29.8 A-c	13.5 A-bc	38.0 A-ab	18.2 A-ab	0.83 A-c
Pooled SD			40	1.1	0.8	0.9	0.7	0.21
1.83 J/cm²		0	1750 A-ab	38.3 A-a	12.0 AB-ab	33.9 B-ab	18.3 A-b	1.50 A-a
		5	1712 A-c	38.5 A-a	12.3 AB-ab	34.2 B-b	19.2 A-a	1.13 A-b
		10	1798 B-b	36.9 A-a	14.8 A-c	34.3 B-b	18.1 C-a	1.53 A-a
		15	1628 B-c	39.5 AB-ab	11.4 A-a	33.2 AB-ab	16.6 A-c	0.79 A-c
		20	1646 B-c	36.2 B-ab	11.4 A-ab	32.1 B-ac	16.8 A-c	0.66 A-c
		25	1638 A-c	34.2 B-ab	13.2 A-bc	31.0 B-c	18.3 A-b	0.79 A-c
		30	1629 A-c	32.6 B-b	12.9 A-ab	30.4 B-c	17.6 A-b	0.82 A-c
Pooled SD			46	1.2	0.9	1.1	0.4	0.10
2.84 J/cm²		0	1715 A-a	39.8 A-a	12.3 AB-a	28.9 C-ab	17.7 A-ab	1.63 A-a
		5	1764 B-b	37.4 A-b	12.7 B-ab	28.2 C-b	18.9 A-c	1.05 A-b
		10	1716 AB-a	37.5 A-b	13.6 A-b	29.2 C-ab	17.5 BC-ab	1.47 A-a
		15	1706 A-a	39.8 B-a	12.7 BC-ab	30.0 BC-a	16.9 A-b	0.93 A-bc
		20	1646 B-c	38.0 C-ab	13.2 B-ab	28.9 C-ab	16.9 A-b	0.70 A-c
		25	1715 A-a	34.2 B-c	12.4 AB-a	26.4 C-c	18.0 A-bc	0.76 A-c
		30	1718 B-a	32.7 B-c	12.3 A-a	26.4 C-c	17.6 A-ab	0.76 A-c
Pooled SD			18	1.1	0.6	0.8	0.5	0.14
3.94 J/cm²		0	1692 A-a	38.4 A-ab	12.3 A-a	26.6 D-a	18.6 A-a	1.31 A-a
		5	1703 A-ab	39.5 A-ab	12.0 A-ab	27.2 C-a	18.4 A-a	1.04 A-b
		10	1747 AB-b	40.2 A-a	13.5 A-c	26.8 D-a	16.9 AB-bcd	0.75 A-c
		15	1673 AB-a	40.9 B-a	13.1 C-bc	26.3 C-a	16.8 A-cd	0.96 A-bc
		20	1705 A-ab	39.4 D-b	13.1 B-bc	24.7 D-b	16.5 A-d	0.71 A-c
		25	1670 A-a	38.1 C-bc	11.6 A-a	22.9 D-c	18.2 A-ab	0.73 A-c
		30	1679 AB-a	36.9 C-c	12.4 A-abc	22.8 D-c	18.0 A-abc	0.74 A-c
Pooled SD			24	0.8	0.6	0.7	0.7	0.15

Results were presented as "means ± standard error" (n=3).
Values in the same columns with different uppercase letters (A-D) indicate significant differences ($p \leq 0.05$) between treatments for the same time of storage.
Values with different lowercase letters (a-d) indicate significant difference ($p \leq 0.05$) within each treatment through storage for each compound. SD=Standard deviation.

Table 2. Organics acids and individual flavonoids content of untreated and UV-C treated grapefruit juices during 16 days refrigerated storage at 10 °C.

			Organic Acids (mg/ 100 mL)				Flavonoids (mg/100 mL)	
Treatment	**T (°C)**	**Days**	**Citric**	**Malic**	**Tartaric**	**Ascorbic**	**Naringin**	**Neohesperidin**
untreated	10	0	1628 A-a	42.7 A-a	48.9 A-a	56.9 A-a	25.6 A-ab	1.60 A-a
		4	1591 A-a	37.6 A-b	41.5 A-c	57.0 A-a	26.7 A-a	2.18 A-ab
		8	1645 AB-a	38.8 A-ab	41.0 AB-c	56.7 A-a	24.8 AB-b	1.56 A-ab
		12	1596 A-a	38.9 A-ab	43.3 A-bc	56.9 A-a	24.4 A-b	1.55 A-ab
		16	1624 AB-a	42.0 A-ab	42.2 A-c	56.9 A-a	26.2 A-ab	1.06 A-b
Pooled SD			37	2.8	2.3	0.8	0.9	0.41
1.83 J/cm²		0	1584 A-a	41.1 A-a	39.1 C-a	49.9 B-ab	26.5 AB-a	1.62 A-a
		4	1670 AB-b	43.3 B-abc	32.4 B-c	50.8 B-abc	26.5 A-ab	1.75 A-a
		8	1625 A-ab	41.8 AB-ab	40.6 AB-a	49.3 B-b	25.0 AB-b	1.98 A-a
		12	1664 BC-b	41.5 AB-a	45.9 A-b	52.3 B-ac	24.4 A-ab	1.57 A-ab
		16	1508 A-a	44.4 BC-bc	39.9 A-a	51.8 B-ac	25.8 A-ab	1.03 A-b
Pooled SD			36	1.6	1.8	1.4	0.7	0.28
2.84 J/cm²		0	1639 A-a	40.1 A-a	39.7 BC-a	45.6 C-a	27.6 A-a	2.15 A-ab
		4	1731 B-c	45.7 B-b	34.8 B-b	48.2 C-a	25.4 A-c	2.42 A-a
		8	1704 B-bc	44.6 B-b	36.0 A-ab	47.2 B-a	23.4 B-d	1.90 A-bc
		12	1694 C-bc	45.9 B-b	37.5 B-ab	47.8 C-a	25.5 AB-c	1.47 A-c
		16	1665 B-ab	46.0 C-b	34.7 A-b	45.9 C-a	26.5 AB-b	0.90 A-d
Pooled SD			30	1.1	2.2	1.7	0.4	0.32
3.94 J/cm²		0	1615 A-a	42.6 A-a	45.1 AB-a	42.1 D-a	27.7 A-a	2.13 A-a
		4	1597 A-a	46.2 B-a	42.1 A-ab	44.3 D-a	28.0 B-b	1.8 A-ab
		8	1607 A-a	43.5 B-a	42.5 B-a	44.2 C-a	26.3 A-ab	1.63 A-ab
		12	1607 AB-a	42.9 AB-a	44.3 A-a	43.4 D-a	25.9 B-b	1.07 A-b
		16	1639 AB-a	42.8 AB-a	39.2 A-b	45.9 C-a	27.2 B-ab	0.80 A-c
Pooled SD			36	1.5	1.4	1.4	1.0	0.34

Results were presented as "means ± standard error" (n=3).
Values in the same columns with different uppercase letters (A-D) indicate significant differences ($p \leq 0.05$) between treatments for the same time of storage.
Values with different lowercase letters (a-d) indicate significant difference ($p \leq 0.05$) within each treatment through storage for each compound.
SD=Standard deviation

Table. 3. Main physicochemical parameters quality, ΔE*, total phenols and EC 50% of untreated and UV-C treated grapefruit juices during 30 days refrigerated storage at 4 °C.

Treatment	T (°C)	Days	pH	°Brix	Titratable acitidy (g citric acid/ 100 mL)	ΔE*	Total Phenols (mg GAE/ 100 mL)	EC50% (DPPH•)
untreated	4	0	3.2 A-ab	9.7 A-a	1.6 A-ab	----	73.0 A-ab	0.0026 A-ab
		5	3.1 A-a	9.6 A-ab	1.6 A-bc	0.3 A-a	79.8 A-c	0.0026 A-ab
		10	3.2 A-ab	9.6 A-ab	1.6 A-c	0.4 A-b	70.7 A-bd	0.0024 A-ab
		15	3.1 A-a	9.5 A-b	1.5 AB-a	0.7 A-b	68.9 A-d	0.0022 A-a
		20	3.3 A-b	9.6 A-ab	1.5 A-a	0.7 A-b	74.0 A-b	0.0023 A-ab
		25	3.2 A-b	9.6 A-ab	1.5 A-a	0.8 A-c	68.6 A-d	0.0027 A-ab
		30	3.1 A-a	9.6 A-b	1.5 A-a	0.1 A-c	63.0 AB-e	0.0028 A-b
Pooled SD			0.1	0.1	0.1	0.3	1.6	1.98E-04
1.83 J/cm²		0	3.2 A-ab	9.8 AB-a	1.6 A-ab	0.4 A-a	69.6 A-a	0.0031 B-ab
		5	3.1 A-bc	9.5 A-b	1.6 A-b	1.6 B-b	76.5 B-b	0.0030 B-ab
		10	3.3 A-b	9.7 AB-ab	1.6 A-b	1.1 A-b	69.5 A-a	0.0032 B-ab
		15	3.3 A-c	9.7 B-ab	1.5 B-ab	0.6 B-b	66.0 AB-ac	0.0031 B-ab
		20	3.2 A-b	9.6 A-b	1.5 B-a	1.0 B-a	69.4 AB-a	0.0028 AB-a
		25	3.2 A-b	9.6 A-b	1.5 B-ab	1.1 B-c	62.6 B-c	0.0031 A-ab
		30	3.1 A-bc	9.7 B-ab	1.6 A-ab	2.0 A-c	61.9 AB-c	0.0034 AB-b
Pooled SD			0.1	0.1	0.1	0.3	2.3	1.95E-04
2.84 J/cm²		0	3.2 A-ab	9.9 AB-a	1.6 A-a	1.0 C-c	72.6 A-a	0.0037 C-a
		5	3.2 A-ab	9.6 A-c	1.6 A-b	1.3 B-a	73.2 C-ab	0.0036 C-a
		10	3.4 A-b	9.6 A-c	1.6 A-b	0.4 B-bc	68.3 A-ab	0.0036 AB-a
		15	3.4 A-a	9.8 B-ab	1.5 AB-a	0.4 B-a	61.3 B-bc	0.0036 BC-a
		20	3.2 A-ab	9.7 A-abc	1.5 A-a	0.9 C-b	60.8 BC-bc	0.0034 AB-a
		25	3.2 A-ab	9.7 A-abc	1.5 A-a	1.1 A-d	59.8 B-b	0.0037 AB-a
		30	3.0 A-a	9.6 AB-bc	1.5 A-a	1.8 A-d	62.1 A-bc	0.0040 BC-a
Pooled SD			0.1	0.1	0.1	0.2	2.4	4.79E-04
3.94 J/cm²		0	3.2 A-abc	9.9 B-a	1.6 A-a	0.3 B-a	69.0 A-ab	0.0042 D-a
		5	3.1 A-ab	9.6 A-c	1.6 A-ab	0.9 C-b	72.9 C-a	0.0042 D-a
		10	3.3 A-c	9.8 B-b	1.6 A-b	0.3 A-a	68.0 A-b	0.0040 B-a
		15	3.0 A-a	9.7 AB-bc	1.5 A-c	0.2 C-c	61.6 B-c	0.0039 C-a
		20	3.2 A-bc	9.6 A-c	1.6 A-a	0.6 C-b	60.8 C-c	0.0040 B-a
		25	3.2 A-bc	9.6 A-c	1.6 A-a	0.8 A-d	60.1 B-c	0.0048 B-a
		30	3.1 A-ab	9.6 AB-c	1.5 A-a	0.8 A-d	59.9 B-c	0.0045 C-a
PSD			0.1	0.1	0.1	0.3	2.1	4.79E-04

Results were presented as "means ± standard error" (n=3).
Values in the same columns with different uppercase letters (A-D) indicate significant differences ($p \leq 0.05$) between treatments for the same time of storage.
Values with different lowercase letters (a-d) indicate significant difference ($p \leq 0.05$) within each treatment through storage for each compound. SD=Standard deviation.

Table. 4. Main physicochemical parameters quality, ΔE*, total phenols and EC 50% of untreated and UV-C treated grapefruit juices during 16 days refrigerated storage at 10 °C.

Treatment	T (°C)	Days	pH	°Brix	Titratable acitidy (g citric acid/ 100 mL)	ΔE*	Total Phenols (mg GAE/ 100 mL)	EC50% (DPPH•)
untreated	10	0	2.9 A-a	11.8 AB-a	2.1 A-a	----	86.1 A-a	0.0025 A-a
		4	2.9 AB-a	11.5 A-ab	2.1 A-a	1.1 A-b	84.4 A-a	0.0021 A-a
		8	2.9 A-a	11.6 A-ab	2.1 A-a	0.8 A-b	85.4 A-a	0.0024 A-a
		12	2.9 A-a	11.3 A-b	2.1 A-a	1.1 A-b	74.8 A-b	0.0022 A-a
		16	2.9 A-a	10.1 A-c	2.1 A-a	2.0 A-c	73.1 AB-b	0.0021 A-a
PSD			0.1	0.1	0.1	0.5	0.1	2.03E-04
1.83 J/cm²		0	2.9 A-a	11.5 C-ab	2.1 A-a	0.7 A-a	86.2 A-a	0.0026 AB-a
		4	2.9 B-a	11.6 A-a	2.1 A-a	1.1 A-b	85.3 A-a	0.0021 A-a
		8	2.9 A-a	11.5 A-a	2.2 B-b	1.0 B-b	81.8 AB-a	0.0026 A-a
		12	2.9 A-a	11.2 A-bc	2.2 B-b	0.8 B-a	77.0 AB-b	0.0022 A-a
		16	2.9 A-a	11.0 B-c	2.2 B-b	1.8 B-c	74.0 AB-b	0.0023 AB-a
PSD			0.1	0.2	0.1	0.3	0.2	2.32E-04
2.84 J/cm²		0	2.9 A-a	11.9 A-a	2.1 A-a	1.7 C-c	86.5 A-a	0.0030 B-a
		4	3.0 A-a	11.8 A-ab	2.0 A-a	1.5 B-bc	89.3 A-a	0.0026 AB-c
		8	2.9 A-a	11.6 A-b	2.1 A-a	1.2 B-a	77.6 B-b	0.0028 A-b
		12	2.9 A-a	11.2 A-d	2.1 A-a	1.4 C-b	71.4 B-c	0.0026 A-c
		16	2.9 A-a	11.4 C-c	2.1 A-a	2.1 A-d	76.4 B-c	0.0026 BC-c
PSD			0.1	0.1	0.1	0.3	0.1	7.10E-05
3.94 J/cm²		0	2.9 A-a	11.7 B-a	2.1 A-a	1.1 B-a	84.2 A-ab	0.0030 B-a
		4	3.0 AB-a	11.6 A-ab	2.1 A-a	1.1 A-a	88.3 A-b	0.0025 B-a
		8	2.9 A-a	11.7 A-b	2.1 A-a	1.6 C-c	80.0 B-a	0.0026 A-a
		12	2.9 A-a	11.2 A-d	2.1 A-a	1.4 C-b	71.8 B-c	0.0024 A-a
		16	2.9 A-a	11.5 C-c	2.1 A-a	2.0 A-d	67.4 A-c	0.0028 C-a
PSD			0.1	0.1	0.1	0.2	0.1	2.95E-04

Results were presented as "means ± standard error" (n=3).
Values in the same columns with different uppercase letters (A-D) indicate significant differences ($p \leq 0.05$) between treatments for the same time of storage.
Values with different lowercase letters (a-d) indicate significant difference ($p \leq 0.05$) within each treatment through storage for each compound. PSD= Pooled Standard deviation.

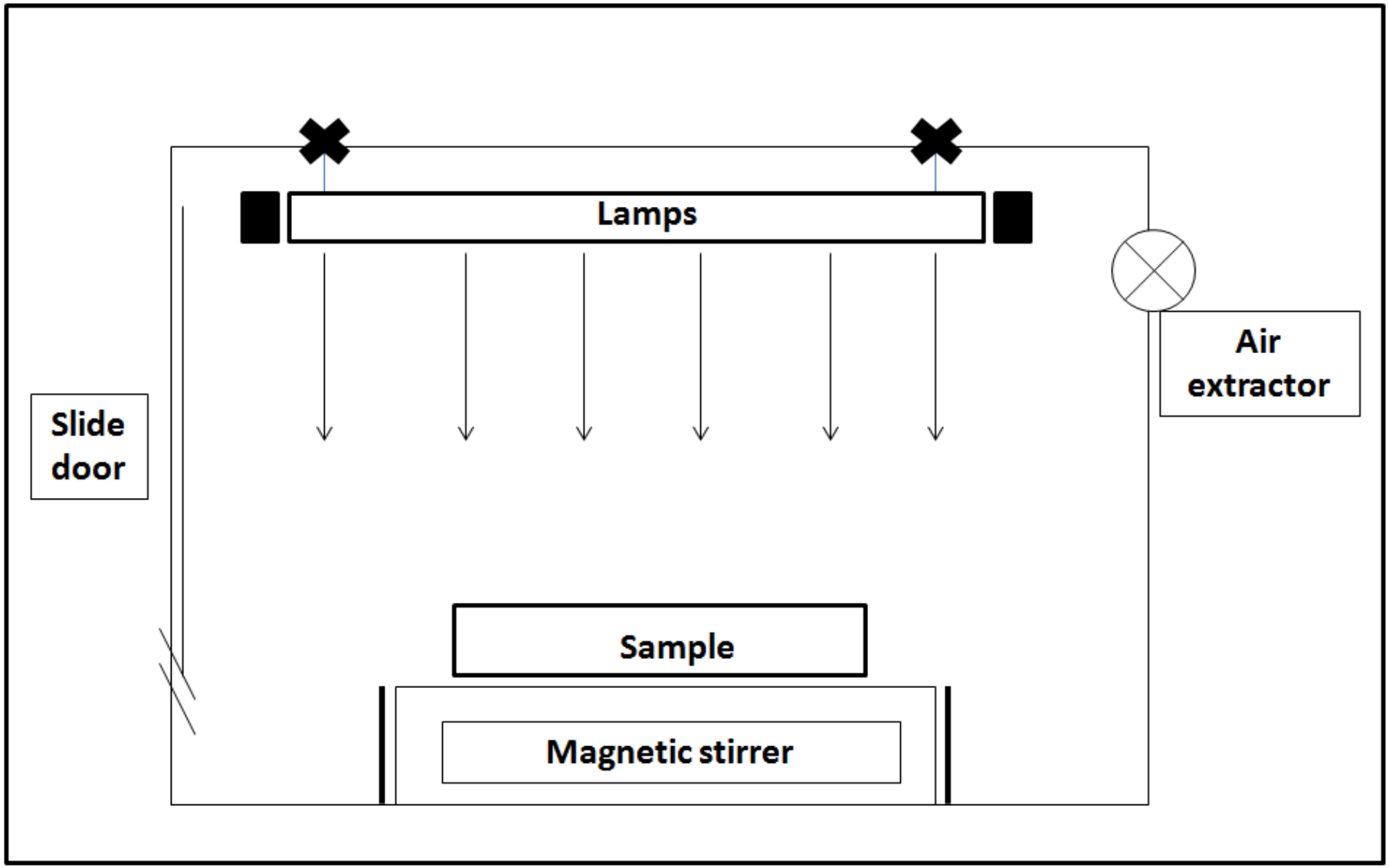

Fig. 1. Diagram of UV-C chamber (not scaled).

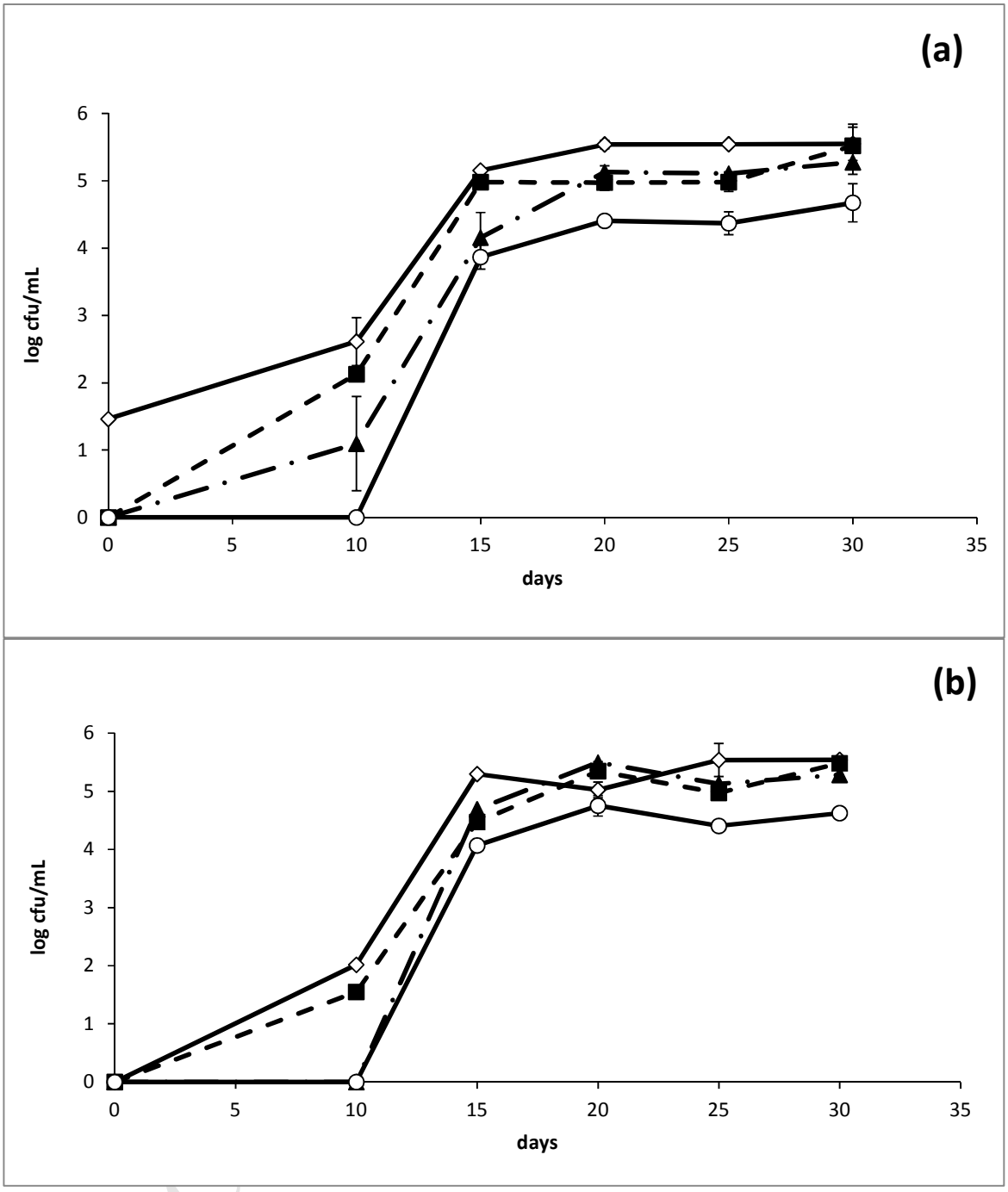

Fig. 2. Changes in total aerobic (a) and yeasts and moulds (b) counts of untreated (◊) and UV-C treated grapefruit juices with 1.83 J/cm^2 (■), 2.84 J/cm^2 (▲) and 3.94 J/cm^2 (○) during 30 days of storage at 4 ºC.

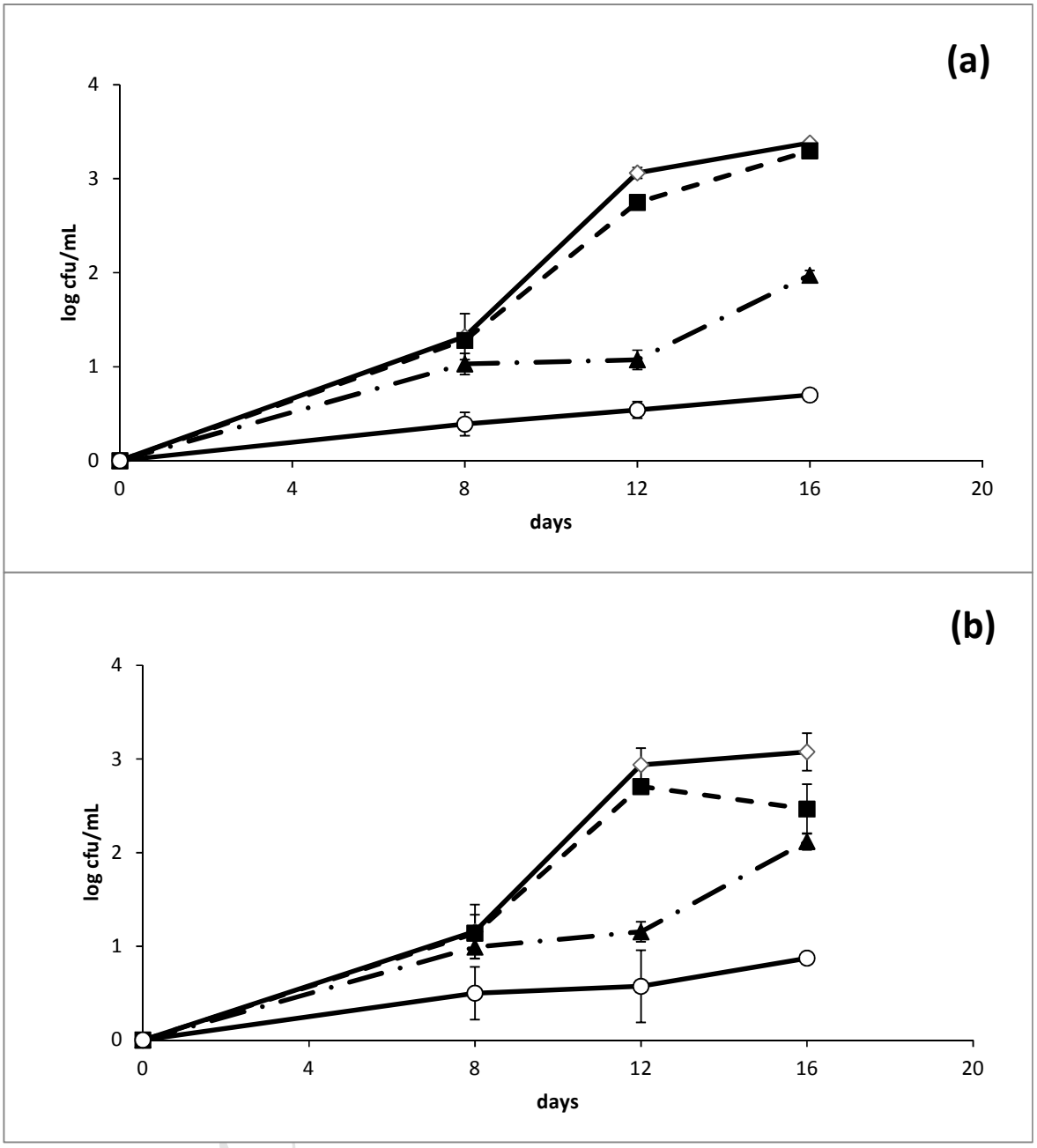

Fig. 3. Changes in total aerobic (a) and yeasts and moulds (b) counts of untreated (◊) and UV-C treated grapefruit juices with 1.83 J/cm^2 (■), 2.84 J/cm^2 (▲) and 3.94 J/cm^2 (○) during 16 days of storage at 10 ºC.