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Inactivation of *Escherichia coli* ATCC 25922 and *Saccharomyces cerevisiae* IMR-R-L 962 in grapefruit [*Citrus paradisi* (Macf.)] juice by UV-C light: changes in bioactive compounds and quality characteristics

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Abstract

The viability of UV-C treatment (0.0-2.36 J/cm²) at 254 nm as a non-thermal preservation process for grapefruit juice on microbial inactivation, the organic acids and individual flavonoids, as well as the quality characteristics (pH, ºBrix, titratable acidity, colour, total phenolics and antioxidant capacity) was evaluated. Additionally, pectin methylesterase (PME) activity was also measured. The effects of UV-C on microbial inactivation were assessed by kinetic studies on the inactivation of inoculated grapefruit juice with one strain of *Escherichia coli* ATCC 25922 and one strain of *Saccharomyces cerevisiae* IMR-R-L 962. The suitability of Weibull distribution and modified Gompertz models was analysed to characterise the UV-C inactivation kinetics for *E. coli* and *S. cerevisiae* in freshly squeezed grapefruit juice. Likewise, the changes after UV-C treatment in citric (CA), malic (MA), ascorbic (AA) and tartaric (TA) acids, as well as naringin (NAR), hesperidin (HES) and neohesperidin (NEO), were quantified by HPLC, whereas the total phenolics and antioxidant capacity ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) were quantified by spectrophotometric methods. Nonlinear inactivation curves were successfully fitted with Weibull-type and modified Gompertz models. However, the Gompertz model allowed a better fit and more accurate estimation of the parameters. UV-C treatment at 1.83 J/cm² achieved a 5.18 ± 0.01 and 2.7 ± 0.15 log CFU/mL reduction in *E. coli* and *S. cerevisiae*, respectively, whereas no significant changes occurred in CA, MA, TA, NAR, HES, NEO, total phenolics, $ABTS^{\bullet+}$, pH, ºBrix, titratable acidity and colour of the grapefruit juices ($p>0.05$). However, PME was partially inhibited and the AA level and $DPPH^{\bullet}$ decreased significantly after treatment, with losses up to 15.9 and 8% (at 1.83 J/cm²), respectively, which were associated with the UV-C dose intensity.

Introduction

Consumer demand for healthy, fresh, minimally processed and preservative-free products is challenging traditional preservation treatments. Thermal treatment constitutes the most extensively available method for inactivation of microorganisms in fruit juices. However, this treatment may cause irreversible losses in nutritional quality and antioxidant activity in the juice, thereby adversely affecting their health-related properties. In this context, non-thermal food preservation processes, without the collateral effect of heat treatments, have been investigated in the last two decades as full or partial alternatives to conventional heat treatment. The use of pulsed electric fields, ultraviolet radiation (UV), ultrasound and high-pressure processing have led to the extension of the term pasteurisation by international microbiological food control agencies. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) report includes UV as an alternative to heat for pasteurisation purposes (JFP Supplement, 2006). The FDA also approves the use of UV light as an alternative to reduce the pathogens in fresh juices. Additionally, several authors have reported the capacity of UV-C light to ensure the microbial safety and maintain the main quality characteristics in juices of orange (Tran and Farid, 2004; Pala and Toklucu, 2013), apple (Noci *et al*., 2008), pomegranate (Pala and Toklucu, 2011), starfruit (Bhat *et al*., 2011) and grape (Falguera *et al.*, 2013). Moreover, UV-C light requires minimal energy compared to thermal pasteurisation. It also removes any traces of pesticides and is not harmful to humans using the equipment or to the environment (Guerrero-Beltran and Barbosa-Canovas, 2005; Koutchma *et al.*, 2009).

In the last 20 years, outbreaks have been

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reported in fruit juices by *Escherichia coli* O157: H7 (enterohaemorrhagic causing colitis and haemolytic uremic syndrome), *Salmonella* spp., *Cryptosporidium parvum* and *Listeria monocytogenes* (CDC, 1996, 1999, 2007). These outbreaks are problematic for processing industries and for public health officials, leading to the development of novel strategies to control the microbiological quality of their products. Several authors suggest the use of *E. coli* ATCC 25922 as a surrogate microorganism of *E. coli* O157:H7 as both strains have similar sensitivity to UV-C radiation in many fruit juices (Duffy *et al*., 2000; Oteiza *et al*., 2005; Pala and Toklucu, 2013).

Moreover, the juices could also be contaminated post-treatment by a variety of spoilage microorganisms and at a high microbial load. These microorganisms have various characteristics, behaviour and responses, according to the conditions of storage and product microenvironment in which they are located. In recent years, three groups of microorganisms have been associated with altered fruit juices: lactic acid bacteria (particularly *Lactobacillus* and *Leuconostoc*) (Keller and Miller, 2006), moulds (*Aspergillus, Paecilomyces* and *Penicillium*) and yeast (genus *Candida* spp., *Saccharomyces* spp. and *Rhodotorula* spp.). Furthermore yeast cells are larger in size than bacteria. The presence of yeast increases the absorption coefficient and these reduce the efficacy of the UV treatment (Oteiza *et al*., 2010).

There is a plethora of evidence that consumption of citrus fruit is generally good for human health and contributes to the prevention of degenerative processes, particularly lowering the incidence and mortality rate of cancer and cardio- and cerebrovascular diseases (Poulose *et al*., 2005). The bioactive compounds present in citrus fruit juice, particularly naringin (NAR) and its aglycone, naringenin, are responsible for these beneficial properties, being commonly incorporated into health supplements. Another bioactive compound present in fruit juice is ascorbic acid (AA), which is one form of vitamin C and is a natural antioxidant that may inhibit the development of major oxidative reactions in humans. These compounds, together with citric (CA), malic (MA) and tartaric (TA) acid contribute to flavour attributes and are used as ‘‘fingerprints’’ to detect juice quality (Cen *et al.*, 2007). Currently, there is a strong demand for technologies that ensure the stability of the bioactive compounds in foods (Lopez-Rubio *et al.,* 2006).

Cloud loss is a major quality defect occurring in cloudy fruit and vegetable juices. This undesired defect is induced by demethylation of pectin by endogenous pectin methylesterase (pectinesterase, PME, EC 3.1.1.11), yielding acidic low-methoxy pectin, which can cross-link with polyvalent cations, such as Ca^{2+}, to form insoluble pectate precipitates (Corredig and Wicker, 2002). To overcome this problem, thermal treatments, such as heating (e.g. 90ºC for 1 min, for citrus juices) or freezing can respectively be used to inactivate PME or slow down its activity. Some studies showed conflicting effects of UV-C processing on the activity of enzymes (Tran and Farid, 2004; Noci *et al.*, 2008; Falguera *et al.*, 2011), reflecting a lack of understanding and the need for more comprehensive studies in citrus fruit juices.

Knowledge of the UV-C doses required to achieve an acceptable reduction in potential pathogens, as well as those that may represent a high risk of spoilage, is a standard requirement for designing a preservation treatment. The physicochemical parameters, such as absorbance, pH, soluble solids (ºBrix) and other components, influence the rate of microbial destruction and could change after processing.

In this context, the current study aimed to analyse the effect of UV-C light (254 nm) on inactivation of *E. coli* ATCC 25922, as a surrogate for *E. coli* O157:H7, and Saccharomyces cerevisiae in inoculated grapefruit juices (GJs) cv. `Duncan´. Additionally, the effect of UV-C light on the levels of CA, AA, MA, TA, NAR, neohesperidin (NEO), and hesperidin (HES), the main physicochemical parameters and PME activity in the GJs were studied.

Materials and Methods

Preparation of GJs and inoculation with E. coli *and* S. cerevisiae

Mature grapefruit (*Citrus paradisi*) cv. ‘Duncan’, with uniform skin colouration, free of cuts, of similar weight and size, and with a 5.5 ratio, were provided by the Experimental Station INTA Bella Vista, Corrientes, Argentina (-28°30`52.43``N, -59°1`47.94``S) were received in the laboratory within 24 h of harvest. The fruits were washed with tap water, sanitised (HClO, 200 ppm, 5 min), rinsed and squeezed with a domestic extractor. Then, the juice was filtered through a sieve (mesh aperture of 3-4 mm) and placed in sanitised conical containers of polypropylene (50 mL) with a screw cap and were immersed in a water bath (Biolec Ind. Argentina) at 90 ± 2°C for 90 s. After that, 18 mL of the fruit juices were cooled and inoculated with 2 mL of *E. coli* ATCC 25922 suspension obtained from the Regional Medical Institute (Universidad Nacional del Nordeste, Argentina) with an inoculum concentration of approximately 1.5 x 10^8 colony forming units (CFU)/mL (0.5 McFarland) to achieve

inoculum concentrations of 1 x 10^7 CFU/mL, and the UV-C treatments were immediately applied. A similar procedure was carried out using *S. cerevisiae* IMR-R-L-962.

UV-C treatments

The UV-C treatments were carried out in a stainless-steel reactor (150 x 100 x 60 cm), equipped with three germicidal lamps for UV-C (TUV 36W/ G36 T8, Philips, Holland) with a peak emission of 254 nm (La Cava and Sgroppo, 2015). The average UV radiation intensity arising from the sample surface was quantified by chemical actinometry (Rahn, 1997). The excess of heat generated inside the chamber was dissipated with a fan, restricting the maximum temperature to 25 ± 1°C. The distance between the surface of the GJs and the lamps was 17 cm and the doses used were 0.28, 0.56, 0.9, 1.83 and 2.36 J/cm², corresponding to 1.5, 3, 5, 9 and 12 min, respectively. Aliquots of the GJ (20 mL) were dispersed in sterile petri dishes (square area of 78.5 cm²), forming a film thickness of 5-7 mm and maintained in suspension using constant magnetic stirring (Precytec AE-29, Argentina). The inoculated GJ was immediately irradiated with UV-C light by placing the samples over the centre line of the tray inside the chamber.

Microbial enumeration

The *E. coli* ATCC 25922 counts were determined by performing serial dilutions (10^{-1}-10^{-6}) of treated (with UV-C), untreated (without UV-C) and control (without inoculated microorganism) GJs with sterile 0.1% peptone water on plate count agar (Britania, Argentina) using a pour plate method. The duplicate plates were incubated at 35 ± 2°C for 48 h. The *S. cerevisiae* IMR-R-L-962 count was carried out on yeast extract, potato dextrose agar (Britania, Argentina) at the same dilutions, at 25°C for 5 d, also using the pour plate method. The results were expressed as log CFU per mL (log CFU/mL) (AOAC, 2000).

Mathematical modelling

Microbial inactivation data were fitted with the cumulative form of a Weibull-type distribution of resistances (Peleg and Cole, 1998):

$$S(t) = \log\left(\frac{N}{N_0}\right) = -b.t^n$$

where S(t) is the fraction of survivors at a given time, and b and n are the scale and the shape parameters, respectively. The b value in the Weibull distribution function represents the rate of inactivation of the cells; while n indicates the concavity of the survival curve (n>1 indicates a downward concavity and n<1, an upward concavity. A log-linear shape is a special case when n=1).

Other statistical parameters (distribution mode, tm; mean, ; variance, ; and coefficient of ''skewness'', ν) were calculated from the following equations (Peleg and Cole, 1998):

$$t_m = \left(\frac{n-1}{nb}\right)^{\frac{1}{n}}$$

$$\bar{t} = \frac{\Gamma\left(\frac{n+1}{n}\right)}{b^{\frac{1}{n}}}$$

$$\sigma^2 = \frac{\Gamma\left(\frac{n+2}{n}\right) - \left[\Gamma\left(\frac{n+1}{n}\right)\right]^2}{b^{\frac{2}{n}}}$$

$$\nu_1 = \frac{\mu_3}{\mu_3^{\frac{3}{2}}}$$

where Γ is the gamma function. The distribution mode, t_m, represents the treatment time at which the majority of the microbial population dies or is inactivated. The mean, $\bar{t}$,corresponds to the inactivation time on average with its variance, σ^2 . The ''skewness'' coefficient, ν, represents the skew of the distribution.

Inactivation curves were also fitted by the modified Gompertz equation (Linton *et al*., 1996) as a function of dose (J/cm²), using the following equation:

$$log\left(\frac{N}{N_0}\right) = \gamma * exp^{(exp^{\alpha+\beta*tiempo})} - \gamma * exp^{(-exp^{\alpha})}$$

where the three parameter estimates (α, β and γ) represent the different regions of the survival curve: the initial shoulder (α, dimensionless), the maximum slope of the survival curve (β, (cm²/J)), and the overall change in the survivor number (γ, dimensionless).

The models performances were evaluated using the root mean square error (RMSE), Akaike's information criterion (AIC) and the Bayesian Schwarz information criterion (BIC).

Content of organic acids

The determination of tartaric (TA), malic (MA), ascorbic (AA) and citric (CA) acid was carried out by the method of Scherer *et al.* (2012). The organic acids content were quantified by high performance liquid chromatography (HPLC) (Shimadzu LC- 10A, Tokyo, Japan) coupled with UV-visible diode array detector (Shimadzu, SPD-M20A, Tokyo, Japan) fixed at 210 nm for TA, MA and CA and 254 nm for AA. A Hypersil ODS column 250 x 6.4 mm, 5

um particle size (Thermo Scientific, Whatman, MA, USA) was used and 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 results were expressed as mg/100 mL GJ 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).

The samples were prepared with 5 mL of GJ mixed with equal parts of mobile phase and filtered through a 0.45 µm nylon membrane previously to injection of 20 µL.

Separation and quantification of flavonoids

Five mL of GJ 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 was filtered through a membrane filter after cooling. Twenty µL of this solution was injected into the HPLC (Shimadzu LC-10A, Tokyo, Japan) coupled with a UV-visible-diode array (Shimadzu, SPD-M20A, Tokyo, Japan) detector fixed at 280 nm for naringin (NAR), hesperidin (HES) and neohesperidin (NEO). A Hypersil ODS column 250 x 6.4 mm, 5 µm particle size (Thermo Scientific, Waltham, MA, USA) was used and 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 GJ 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 in a ratio 20:80.

Main physicochemical parameters

The 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 GJ.

Color

The color of the fresh and treated GJ 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 GJ c at the beginning of the experiment.

Total phenols, antioxidant activity (AOC)

The total phenolic (TP) content and antioxidant capacity ($DPPH^•$ and $ABTS^{•+}$ assays) were determined with the microplate protocol proposed by Magalhaes *et al.* (2012) using dilutions of GJ in water and spectrophotometric detection (Multiskan GO, Thermo scientific Instruments). The results were expressed as mg of gallic acid equivalents (GAE), % inhibitions and TEAC (Trolox equivalent antioxidant capacity) per 100 mL of GJ for TP, $DPPH^•$ and $ABTS^{•+}$ assays respectively.

Pectin methylesterase (PME) assay

PME activity was tested by continuous titration of carboxyl groups released from a pectin solution using an automatic pH-meter (Metrohm meter pH-/ ion, Switzerland) and 0.01N NaOH. Routine assays were performed with 3.5 mg/mL citrus pectin solution (degree of esterification of 75%, 30 mL) containing 0.117 M NaCl (pH 7.0) at 22.5ºC. The activity unit (U) of PME was defined as the amount of enzyme required to release 1 µmol of carboxyl group per minute, under the above-mentioned conditions.

$$\text{PME (U/mL)} = \frac{(\text{mL NaOH}) * (\text{NaOH Normality})}{(\text{reaction time}) * (\text{mL sample})} \quad (2)$$

The relative PME activity was calculated using the following equation:

$$\text{Relative PME activity (\%)} = \frac{(\text{PME of treated sample})}{(\text{PME of untreated sample})} * 100 \quad (3)$$

Statistical analysis

The result of each determination was expressed as the mean of 3 determinations. The pooled standard deviation was performed to each parameters analyzed. Significant differences were evaluated by ANOVA and Duncan test ($p < 0.05$) using the Info-Stat Statistical Software (Cordoba-Argentina, 2009).

Results and Discussion

Effect of UV-C on E. coli *and* S. cerevisiae

Non-linear, semi-logarithmic survival curves for *E. coli* ATCC 25922 and *S. cerevisiae* IRM-R-L-962 in GJ treated by UV-C light were characterised by the Weibull distribution of resistance and modified Gompertz model (Figure 1). The *E. coli* inactivation curve exhibited n values around 1, as expected for a linear response. In contrast, *S. cerevisiae* showed n<1, approximating a pronounced sigmoidal curve. Interestingly, n<1 indicated that the remaining cells were more resistant to the treatment, whereas n>1,

Table 1. Decimal reduction dose (D10), Weibull model parameters (b and n) and related statistics and Gompertz model parameters (α, β and γ) a corresponding to *E. coli* and *S. cerevisiae* survival in grapefruit juice treated with UV-C light.

	First-order model		Weibull model							Gompertz model			
Strain	D_{10} (J/cm²)	R^2_{adj}	b (min^{-1})	N (-)	R^2_{adj}	t_m (min)	$\bar{t}$ (min)	σ_t^2 (min^2)	v (-)	α (-)	β (min^{-1})	γ (-)	R^2_{adj}
E. coli	0.36 (p<0.001)	0.99	0.48 (p=0.002)	1.05 (p<0.001)	**0.99**	0.11	2.17	4.73	2.01	8.97 (p<0.001)	1.28 (p=0.001)	0.2 (p=0.001)	0.99
S. cerevisiae	0.53 (P=0.036)	0.87	0.56 (p=0.047)	0.72 (p=0.011)	0.93	-	2.76	15.27	1.26	3.2 (p=0.001)	2.54 (p=0.063)	0.61 (p=0.053)	**0.97**

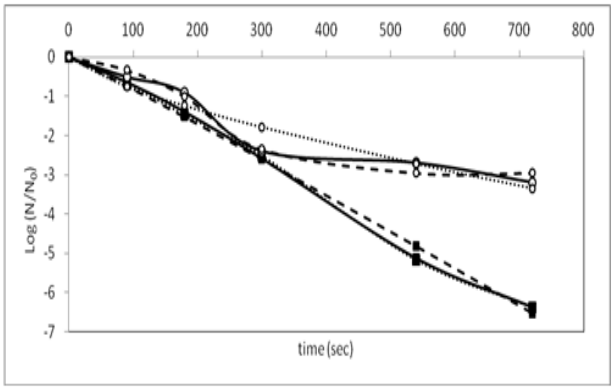

Figure 1. Experimental survival curves (solid line) and fitted values derived from Weibull (points) and Gompertz (dashed line) models for *E. coli* (■) and *S. cerevisiae* (o) treated with UV-C light.

suggested an accumulation of the lethal effect, resulting in an increasing rate of destruction with time. Furthermore, the scale factor (b), which is considered to be a measure of microorganism resistance to treatment (kinetic parameter) (Pina-Pérez *et al.*, 2009), was lower for *E. coli* than *S. cerevisiae.* In agreement with these findings, the decimal reduction doses (D10) obtained through the first-order model proved that in GJ, *E. coli* was more sensitive to UV-C light compared to *S. cerevisiae* (Table 1).

The goodness-of-fit criteria for Weibull and Gompertz demonstrated that both models constituted good alternatives to quantify the microbial response to UV-C light (R^2_{adj} =0.93–0.99) (Table 1). However, based on the RMSE, AIC and BIC values, the modified Gompertz model showed the best performance in most instances. Interestingly, the survival curve obtained for *E. coli* could be treated as a linear one (R_{2adj}=0.99).

Treatment with UV-C light for 9 min (1.83 J/cm²) decreased *E. coli* ATCC 25922 and *S. cerevisiae* IRM-R-L-962 counts by 5.18 ± 0.01 and 2.7 ± 0.15 log CFU/mL, respectively. Similarly, Pala and Toklucu (2013) reported an *E. coli* ATCC 25922 decrease of 5.72 log CFU/mL in inoculated orange juices,

Table 2. Minimum RSME, AIC and BIC values for the survival curves of the assayed microorganisms in UV-C treated grapefruit juice.

	RSME		AIC		BIC	
Strain	Weibull	Gompertz	Weibull	Gompertz	Weibull	Gompertz
E. coli	0.23	0.01	3.06	-11.10	1.89	-11.93
S. cerevisiae	0.44	0.29	9.39	3.52	8.22	2.68

after 3 passes through a UV-C reactor, while it was completely inactivated after 5 passes. Koutchma *et al.* (2007) reported a 3.1 log reduction of *E. coli* K12 cells in a model caramel solution, which simulates orange juice after 3 passes through a UV-C reactor with 24 lamps. Moreover, Donahue *et al*. (2004) reported a minimum 5 log decline in *E. coli* ATTC 25922 populations in apple juice treated with 35 mJ/cm² UV-C. Caminiti *et al.* (2012) found 2.66 J/cm² UV-C resulted in a 4.59 log reduction in *E. coli* in apple juice. The good efficiency of the equipment used in this work is based on the agitation rate that is produced during treatment (through a magnetic stirrer) and the thickness of the juice during processing (5-7 mm). Oteiza *et al.* (2005) demonstrated that the germicidal effect on two *E. coli* strains is related, among other factors, with the agitation rate and the thickness of the layer juice.

Guerrero-Beltran *et al*. (2009) reported similar inactivation results for *S. cerevisiae* using a commercial system (UV Atlantic, Co., Hauppauge, NY). After UV-C irradiation at a flow rate of 1.02 L/min for 30 min, the yeast population decreased by 2.42 log CFU/mL in pink-pale GJ. However, in mango nectar, a reduction of 2.7 log CFU/mL was achieved at a flow of 0.45 L/min (Guerrero-Beltran and Barbosa-Canovas, 2006). The UV decimal reduction time (Duv) for *E. coli* (0.36 J/cm²) was almost 0.6 times lower than *S. cerevisiae* (0.53 J/cm²). These results could be due to the morphological differences between these two microorganisms.

Table 3. Citric, ascorbic, malic and tartaric acid and individual flavonoids of untreated and UV-C treated grapefruit juices. Results were presented in mg/ 100 mL of juice.

UV-C (J/cm²)	Citric acid	Ascorbic acid	Malic acid	Tartaric acid	Naringin	Neohesperidin	Hesperidin
0.00	1115 ± 38^{a}	49.1 ± 0.8^{a}	18.2 ± 1.2^{a}	42.4 ± 2.6^{c}	26.8 ± 1.8^{a}	2.0 ± 0.1^{a}	5.3 ± 5.3^{a}
0.28	1157 ± 16^{a}	48.4 ± 0.8^{a}	18.7 ± 1.9^{a}	50.9 ± 1.1^{a}	26.9 ± 1.4^{a}	2.0 ± 0.2^{a}	5.1 ± 5.1^{a}
0.56	1150 ± 10^{a}	46.3 ± 0.3^{b}	18.1 ± 1.8^{a}	41.1 ± 2.4^{c}	25.7 ± 0.1^{a}	1.9 ± 0.0^{a}	4.5 ± 4.5^{a}
0.90	1065 ± 27^{a}	44.7 ± 0.8^{b}	19.6 ± 1.4^{a}	42.9 ± 0.3^{c}	25.8 ± 0.9^{a}	1.7 ± 0.2^{a}	5.3 ± 5.3^{a}
1.83	1153 ± 39^{a}	41.3 ± 1.6^{c}	17.8 ± 1.0^{a}	43.9 ± 1.0^{bc}	25.2 ± 0.2^{a}	1.9 ± 0.1^{a}	5.2 ± 5.2^{a}
2.36	1160 ± 50^{a}	39.5 ± 0.6^{d}	19.1 ± 1.2^{a}	47.7 ± 3.9^{ab}	25.1 ± 0.4^{a}	1.9 ± 0.2^{a}	5.0 ± 5.0^{a}

Column values with different lowercase letters (a, b, c and d) indicate significant differences ($P < 0.05$). Results were presented as means (n = 3) ± standard deviation.

Yeasts and moulds are more resistant than other bacteria, probably due to their DNA structure and the chemical composition and thickness of the cell wall (Tran and Farid, 2004).

Organic acids

Citrus fruits are a good source of organic acids such as AA and CA. These two organic acids collectively contribute to the taste and health benefits of citrus fruits (Cen *et al.,* 2007). At 1134 ± 37 mg/100 mL, CA was the predominant organic acid in GJ, while MA and TA contents were 18.6 ± 0.7 and 44.8 ± 3.7 mg/100 mL, respectively (Table 3). After UV-C treatment the CA and MA levels remained unchanged ($p<0.05$), which was observed in UV-C-treated orange juice (Pala and Toklucu, 2013). The initial AA content in GJ cv. 'Duncan' was 49.03 + 0.76 mg/100 mL, in concurrence with the values reported by Igual *et al*. (2010) and Uckoo et al. (2013). The AA content in GJ was significantly reduced by UV-C treatment ($p<0.05$) compared to the untreated sample and the decrease was dose-dependent. Losses of 1.5, 5.8, 9.0, 15.9 and 19.5% were observed after 0.28, 0.56, 0.9, 1.83 and 2.36 J/cm² irradiation, respectively. These results were in agreement with Tran and Farid (2004) and Pala and Toklucu (2013), which reported AA losses of 17 and 16.6% after high UV-C irradiation of orange juice. A linear correlation was observed between the semi-log of the AA concentration (mg/100 mL) and the UV-C dose (J/cm²). The slope of the graph was -0.0937, R^2 =0.9924 and the data fitted a first-order model ($p<0.001$) (Figure not shown), with no lack-of-fit evident, indicating a strong relationship between the UV-C dose and AA degradation in GJ. Tikekar *et al.* (2011) suggested that the mechanism for UV-induced AA degradation in juices is similar to the general mechanism for metal-catalysed oxidation. Moreover, the decrease in the AA content could be associated with the coincidence between its absorption maximum and the peak emission of UV-C lamps.

Flavonoids

Grapefruits are a good source of flavanones, a group of flavonoids that are unique to citrus species and associated with numerous health promoting properties (Peterson *et al*., 2006). NAR is the flavonoid compound found in grapefruit that gives its characteristic bitter flavour. NAR and its aglycone (naringenin) are commonly used in health supplements. The flavonoid detected in highest amounts in the GJ was NAR at 26.8 ± 1.6 mg/100 mL, whereas the NEO and HES content were ten- and five-fold lower, respectively (Table 3). The NAR, NEO and HES contents were very similar to those reported by Uckoo *et al.* (2013) and Igual *et al.* (2011). Previously, UV-C treatment demonstrated no significant effects on the levels of these bioactives ($p>0.05$), in contrast to thermal treatment, which decreased the presence of all three constituents (Igual *et al*., 2011).

Main physicochemical parameters

The pH and ºBrix values were 3.03 ± 0.17 and 9.5 ± 0.1, and the titratable acidity was 2.09 ± 0.02 g CA/100 mL (Table 4). After the UV-C treatment, no significant changes ($p<0.05$) were observed in any of the parameters analysed. Similar results were observed by other authors in various fruit juices (Noci *et al*., 2008; Bhat R *et al*., 2011; Caminiti *et al*., 2011; Pala and Toklucu, 2012; Falguera *et al*., 2013).

The absorbance coefficient (49.47 cm^{-1}) and turbidity (2500 NTU) of freshly squeezed GJ were determined at 254 nm. These values were comparable to those found in orange and guava juice, respectively (Koutchma et al., 2009).

Colour

Colour is one of the most important criteria for consumer preference and is considered a key parameter of juice quality. White GJ contains

Table 4. Main physicochemical parameters quality, ΔE, total phenols and antioxidant capacity of untreated and UV-C treated grapefruit juices.

UV-C (J/cm²)	pH	Brix	Acidity titratable [A]	ΔE	Total phenols [B]	DPPH [C]	TEAC [D]
0.00	3.20 ± 0.10^{a}	9.5 ± 0.1^{a}	2.07 ± 0.03^{a}	– ±	58.5 ± 6.4^{a}	101 ± 6^{a}	15.5 ± 0.2^{a}
0.28	3.19 ± 0.10^{a}	9.5 ± 0.1^{a}	2.12 ± 0.04^{a}	0.21 ± 0.01^{a}	57.4 ± 2.8^{a}	99 ± 4^{ab}	15.7 ± 0.5^{a}
0.56	3.21 ± 0.06^{a}	9.5 ± 0.1^{a}	2.09 ± 0.03^{a}	0.13 ± 0.05^{a}	62.0 ± 2.5^{a}	98 ± 7^{abc}	15.4 ± 0.1^{a}
0.90	3.15 ± 0.08^{a}	9.4 ± 0.1^{a}	2.10 ± 0.03^{a}	0.45 ± 0.14^{b}	62.6 ± 2.9^{a}	99 ± 1^{ab}	15.4 ± 0.2^{a}
1.83	3.16 ± 0.05^{a}	9.5 ± 0.2^{a}	2.07 ± 0.03^{a}	0.37 ± 0.05^{b}	61.8 ± 2.0^{a}	92 ± 6^{bc}	15.6 ± 0.4^{a}
2.36	3.13 ± 0.05^{a}	9.4 ± 0.1^{a}	2.07 ± 0.03^{a}	0.47 ± 0.05^{b}	62.1 ± 4.0^{a}	88 ± 8^{c}	15.4 ± 0.2^{a}

Column values with different lowercase letters (a, b, c and d) indicate significant differences ($P<0.05$). Results were presented as means (n = 3) ± Standard Deviation.
[A] Expressed as "g citric acid/100 mL"
[B] Expressed as "mg gallic acid/ 100 mL".
[C] Expressed as "% inhibition".
[D] Expressed as "Trolox equivalent antioxidant capacity/ 100 mL".

colourless carotenes, phytoene, and phytofluene, which account for 70-80% of the total content in the peel and pulp. Immediately after UV-C treatment, significant ($p<0.05$) colour differences were detected (Table 4) with dose higher to 0.90 J/cm^2, however these values did not exceed 0.5 ($\Delta E<0.5$). Likewise, negligible colour differences were observed by Caminiti *et al.* (2012) in UV-C-treated apple juice. However, Guerrero-Beltran and Barbosa-Canovas (2009) reported a change of 12.43 ± 0.05 in the total colour of pink-pale GJ after UV-C treatment that was attributed to oxidation of the double bonds in lycopene due to the UV light effect.

Total phenolic compounds and antioxidant activity

The total phenolic compounds were detected at 60.7 ± 1.6 mg/100 mL, which was similar to those reported in other GJ varieties (Igual *et al.*, 2010). The total phenolic compounds content was not significant affected ($p>0.05$) (Table 4) by the UV-C treatment, consistent with observations made in orange juice (Pala and Toklucu, 2012) although in other fruit juices the results were variable (Noci *et al.*, 2008; Falguera *et al*. 2013).

The antioxidant activity was determined by the free radical-scavenging DPPH• and $ABTS^{\bullet+}$ assays and the values were expressed as a percentage of inhibition and TEAC, respectively. After UV-C treatment, the $ABTS^{\bullet+}$ antioxidant activity remained unchanged ($p>0.05$). However, the $DPPH^{\bullet}$ radical scavenging activity decreased significantly compared to the control ($p<0.05$) at doses >1.83 J/cm² (Table 4). This behaviour was similar to the trend observed in the AA content.

PME activity

PME is an enzyme found in citrus juices and responsible for their quality deterioration through an undesirable precipitation of cloud particles. Hence, its inactivation is required during processing. The effect of UV-C radiation on GJ cv. `Duncan' was studied in this work by applying doses ranging from 0-11.8 J/cm². The PME activity showed losses of 19, 37, 39, 42 and 52% after UV-C treatment with 1.83, 2.84, 3.94, 7.9 and 11.8 J/cm², respectively. However, a decrease of >99.9% of the PME activity is needed to avoid `cloud loss' in juice, which can be achieved by heat treatment at 90°C for 60 s. In orange juices treated under a UV-C continuous system, the PME activity only decreased by 5% (Tran and Farid, 2004), whereas in an orange and carrot juice blend it was more noticeable (18%) (Caminiti *et al.*, 2011).

Conclusion

The results obtained in this study confirm the potential application of UV-C treatment in grapefruit processing industries. An irradiation dose of 1.83 J/cm² ensured a 5 log CFU/mL decrease in the target microorganism. Nonlinear inactivation curves were successfully fitted with Weibull-type and modified Gompertz models. Although, based on the RMSE, AIC and BIC values, the modified Gompertz model showed the best performance in both instances (*E. coli* and *S. cerevisiae*). The UV-C treatment induced a slight decrease in the AA content (16%), accompanied by a decrease in antioxidant activity, while other bioactive compounds and quality parameters did not show significant changes. Although the PME was partially inactivated, a cloud juice was observed after 24 h. Further studies are required to optimise the processing parameters, study the native microorganisms present and assess the organoleptic properties of juice for providing consumer preferred

fresh and naturally nutritious GJ.

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