Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice

E. Gayán, M.J. Serrano, S. Monfort, I. Álvarez, S. Condón

PII: S0260-8774(12)00352-4
DOI: http://dx.doi.org/10.1016/j.jfoodeng.2012.07.018
Reference: JFOE 7025

To appear in: *Journal of Food Engineering*

Received Date: 14 March 2012
Revised Date: 11 July 2012
Accepted Date: 17 July 2012

Please cite this article as: Gayán, E., Serrano, M.J., Monfort, S., Álvarez, I., Condón, S., Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice, *Journal of Food Engineering* (2012), doi: http://dx.doi.org/10.1016/j.jfoodeng.2012.07.018

Combining ultraviolet light and mild temperatures for the inactivation of *Escherichia coli* in orange juice.

Gayán, E., Serrano, M. J., Monfort, S., Álvarez, I., Condón, S.*

Tecnología de los Alimentos, Universidad de Zaragoza, C/ Miguel Servet 177, CP 50013, Zaragoza, Spain

* Prof. Santiago Condón. Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, C/ Miguel Servet 177, CP 50013, Zaragoza, Spain.

TEL.: 0034 976 76 15 81

FAX: 0034 976 76 15 90

E-mail: scondon@unizar.es

ABSTRACT

It is difficult to guarantee the effectiveness of UV technology to reach 5 Log_{10} cycles of inactivation of *Escherichia coli* in a large amount of fruit juices with high absorption coefficients and turbidities, such as orange juice. The aim of this work was to overcome this limitation by combining UV light and mild temperatures. UV treatments were carried out in an equipment with eight individual annular thin film flow-through reactors connected sequentially and submerged in a thermostatic water bath. A treatment of 13.55 J/mL reached 0.25±0.04, 0.41±0.13, 0.84±0.32, 0.96±0.12, 2.57±0.05, 5.41±0.23, and more than 6 Log_{10} cycles of inactivation of *E. coli* STCC 4201 suspended in commercial sterilized orange juice at 25.0, 40.0, 50.0, 52.5, 55.0, 57.5, and 60.0 ºC, respectively. The comparison of UV resistance at 25 ºC with heat resistance at mild temperatures demonstrated a synergistic effect of both technologies applied simultaneously. The maximum synergistic lethal effect was reached at 55 º C (68.03%).

A UV light treatment (23.72 J/mL) at 55 ºC (3.6 min) of freshly squeezed orange juice allowed reaching more than 5 Log_{10} cycles of inactivation of a *E. coli* cocktail (STCC 4201, STCC 471, ATCC 27325, ATCC 25922, and O157:H7 Chapman strain). The selected treatment did not affect the pH, acidity, ºBrix, and color, and decreased 16.45% ascorbic acid content and 63.96% the Pectinmethylesterase activity.

Keywords: Pasteurization, orange juice, *Escherichia coli*, ultraviolet, heat treatment

1. Introduction

The food industry has been challenged to improve the safety, stability, and convenience of foods and a great effort has been made to develop more appropriate methods for food preservation and sanitation. At present, it can be foreseen that none of the new technologies under study will replace traditional preserving procedures for a wide range of products. However, each one shows particular advantages that may make it the most adequate technology for a certain product or process.

Among the nonthermal technologies developed in the last few decades, ultraviolet (UV) light irradiation is one of the most promising because it is easy to use and lethal to most microorganisms (Bintsis et al., 2000), it does not generate chemical residues (Guerrero-Beltrán, 2004), and it is a dry cold process (Bachmann, 1975) that can be effective at low cost in comparison with other preservation methods (Geveke, 2005).

The wavelength range for UV light for food processing varies from 100 to 400 nm and is categorized as UV-A (320-400 nm), UV-B (280-320), and UV-C (200-280 nm). Short-wave UV radiation (UV-C) is considered the germicidal region lethal to most types of microorganisms (Bintsis et al., 2000; Sizer and Balasubramaniam, 1999). In this range, the maximum efficacy is best achieved between 250 and 270 nm, and decreases as wavelength increases. UV-C light of 254 nm, generated by low-pressure mercury lamps, is usually used for disinfection purposes.

The use of UV light is well established for air and water disinfection, but its use for the pasteurization of liquid foods is still limited. Evidence has demonstrated that UV technology is a promising alternative to thermal pasteurization for a range of liquid foods. In fact, the National Advisory Committee on Microbiological Criteria for Foods of the USDA (NACMCF) revised the definition of "pasteurization" in 2004 and included UV irradiation as an alternative to heat that can be used for pasteurization

purposes. However, the bactericidal efficacy of UV light depends on the optical properties of the treatment medium. The penetration of UV radiation depends on the absorptivity of the liquid, which varies with the color of compounds and the amount of soluble and/or suspended solids (Koutchma et al., 2004). The penetration depth also depends on the turbidity of the media. Suspended solids increase the absorptivity but are also responsible for reflection and scattering phenomena (Koutchma et al., 2004; Liltved and Cripps, 1999). As a consequence of these phenomena, UV light only penetrates a very short depth inside the liquid food surface. For example, it has been estimated that 90% of the radiation is absorbed in about 1.1 cm depth of wine, 0.63 cm of beer, 0.67 cm of clear apple juice, and 0.1 cm in orange juice (Koutchma, 2009). This explains the wide range of variation of Log_{10} cycles of bacterial inactivation obtained when microorganisms are suspended in liquid foods. Overall, published data demonstrated that it is difficult to guarantee the effectiveness of UV technology to reach 5 Log_{10} cycles of bacterial inactivation in a large amount of fruit juices with high absorption coefficients and turbidities.

To overcome the limitations of non-thermal methods of food preservation, new processes have been designed by combining several technologies applied at lower intensities, but with equivalent or even higher degrees of stability and safety. Today some combinations with UV light have been explored with pasteurization purposes. It has been demonstrated that UV light followed by pulsed electric fields (PEF) treatments have an additive effect (Gachovska, 2008; Noci et al., 2008). Also Walkling-Ribeiro (2008) found an additive effect when processing apple juice by a hurdle sequence including UV light, pre-heating, and PEF. More promising is the combination of UV, laser, and microwave radiation or conventional heating applied successively, because they act synergistically (Maktabi et al., 2011). The simultaneous application of heat at

96 sublethal temperatures and non-thermal technologies, such as high hydrostatic
97 pressures, ultrasounds, and PEF have been thoroughly investigated (Raso and Barbosa-
98 Canovas, 2003). Surprisingly there are very scarce data in literature about the lethal
99 effect of UV light at midrange temperatures. Previously, Geveke et al. (2008) indicated
100 the improvement of UV lethal effect at mid-temperatures (40-50 ºC) in liquid egg white.
101 Ukuku and Geveke (2010) investigated a combined treatment of UV light and radio
102 frequency electric field for the inactivation of *E. coli* in apple juice. From their results it
103 could be deduced that the inactivating effect of UV light increased with temperature in
104 the range of 20 to 40 ºC. More recently, Gayán et al. (2011) demonstrated that the
105 temperature barely changed the UV resistance of *E. coli* up to 50.0 ºC. Above this
106 threshold value inactivation rates due to the combined process synergistically increased
107 up to 60.0 ºC. This data, obtained in laboratory media, suggested the possibility of
108 design-combined processes for the pasteurization of fruit juices with high absorption
109 coefficients.
110 The objective of this investigation was 1) to determinate the lethal effect of UV-C
111 treatments at mild temperatures on the UV-C resistant *E. coli* STCC 4201 strain
112 suspended in sterilized orange juice; 2) to establish the optimum temperature for the
113 combined process; and 3) to evaluate the effect of the optimized treatments on a cocktail
114 of five strains of *E. coli* on fresh squeezed orange juice and on the quality of the juice.
115 Orange juice was selected as treatment media because of its high absorption coefficient.
116

117 **2. Materials and methods**

118 **2.1. Bacterial culture and media**

119 The strains of *E. coli* STCC 4201 and STCC 471 were provided by the Spanish Type
120 Culture Collection (STCC). The strain of *E. coli* ATCC 25922 and ATCC 27325 was

provided by the American Type Culture Collection (ATCC). The strain *E. coli* O157:H7 used in this investigation is a VTEC- (Phage type 34) isolated by Dr. Chapman (Chapman et al., 1993). The bacterial cultures were frozen at -80 ºC in cryovials. Stationary-phase cultures were prepared by inoculating 10 mL of tryptone soy broth (Biolife, Milan, Italy) supplemented with 0.6% (w/v) yeast extract (Biolife) (TSBYE) with a loopful of growth from tryptone soy agar (Biolife) supplemented with 0.6% (w/v) yeast extract (TSAYE). The precultures were incubated at 35 ºC for 6 h, in a shaking incubator. 50 µL of the precultures were inoculated into 50 mL of fresh TSBYE and incubated for 24 h under the same conditions, which resulted in stationary-phase cultures containing approximately 2×10^9 CFU/mL.

2.2. Treatment media

To determinate the lethal effect of UV-C treatments at mild temperatures on *E. coli* STCC 4201, and to establish the optimum temperature for the combined process, commercial sterilized orange juice was used as treatment media. The effect of the optimized treatments on a cocktail of five strains of *E. coli* and on the quality of the juice was evaluated with fresh squeezed orange juice.

Sterilized orange juice (García Carrion S.A., Spain) was purchased from a local market. It showed an absorption coefficient of 81.10 cm^{-1}, and a turbidity of 4,460 nefelometric turbidity units (NTU). Fresh squeezed orange juice was prepared by squeezing (Zumex, versatile 230 V, Valencia, Spain) Valencia variety orange fruits, and filtering the juice through a stainless filter with net square holes of 1 mm^2. The filtered juice showed an absorption coefficient of 51.52 cm^{-1} and a turbidity of 3,075 NTU)

146 **2.3. UV treatments**

147 UV treatments were carried out in the equipment previously described (Gayán et al.,
148 2011). The whole system consisted of eight individual annular thin film flow-through
149 reactors connected sequentially. Each reactor included a low pressure UV lamp (TUV
150 8WT5, Philips, U.S.A.) with a length of 282.3 mm and 8 W of total power, emitting
151 90% of energy at a wavelength of 254 nm, fixed at the axis of an outer glass tube (25
152 mm of inner diameter) and enclosed by a quartz tube (20 mm of outer diameter) to
153 prevent direct contact of the lamp with the treatment medium. In the annular gap (2.5
154 mm) a stainless steel coil spring was installed to improve the turbulence of the flow.
155 Outside and inside coil diameters of the spring were 23 and 25 mm, respectively, and its
156 length and pitch were 270 mm and 10 mm, respectively. The equipment also included a
157 feed tank, a peristaltic pump (Ismatec, mod. ISM 10785, Glattbrugg, Switzerland), a
158 heating/cooling coil exchanger and extraction valves at the outlet of each reactor. The
159 circuit and reactors were submerged in a 90 L water bath heated at different
160 temperatures (25, 40, 50, 52.5, 55, 57.5 or 60 ºC) by the circulating water of a
161 peripheral thermostatic bath (Huber, mod. Kattebad K12, Offenburg, Germany). Two
162 thermocouples (Almemo, mod. ZA 020-FS, Bernburg, Germany) fitted to the input of
163 the first and the outlet of the last reactor allowed the control of the treatment medium
164 temperature.

165 The applied doses were calculated dividing the radiant power (W) by the flow rate (Q)
166 as it has been previously described (Geveke 2005; Keyser et al. 2008). The flow rate
167 was kept constant at 8.5 L/h. Previous experiments demonstrated that above this value,
168 the efficacy of the treatments was hardly affected by the flow rate (Gayán et al., 2011).
169 With this flow rate the mean residence time in the equipment was 3.6 min. When heat
170 and UV treatments were compared, the UV treatment times were calculated dividing the

mean residence time (3.6 min.) by 8 (number of reactors) and multiplying by the number of reactors that the sample goes through before it was removed. The UV applied dose at the outlet of successive reactors was: 3.4, 6.8, 10.2, 13.5, 16.9, 20.3, 23.7 and 27.1 J/mL respectively.

The medium to be treated was added with the bacterial suspension to achieve 10^7-10^8 CFU/mL and pumped through the heat exchanger to the reactors. When the treatment conditions were stabilized, samples were withdrawn through the sampling valves at the outlet of each reactor and 0.1 mL or 1 mL was immediately pour plated in the recovery media.

2.4 Heat treatments

Heat treatments were carried out in a specially designed resistometer (Condón et al., 1993). The thermoresistometer TR-SC is a mixing method designed for studying heat inactivation kinetics by the multipoint method. Briefly, the instrument consists of a 400 mL vessel provided with an electrical heater for thermostation, an agitation device to ensure inocula distribution and temperature homogeneity, and ports for injecting the microbial suspension and for the extraction of samples. 350 mL of orange juice were placed in the vessel of the TR-SC and heating was turned on. Once the juice reached the preset temperature (T±0.05 °C) (56, 58, 60 and 62 ºC), it was inoculated with 0.2 mL of an adequately diluted microbial cell suspension. After inoculation, 0.2 mL samples were collected at different heating times and immediately pour plated. Before inoculation bacterial cells were pre-adapted to the heating media by suspending 0.1 mL of grown culture into 0.9 mL of orange juice and incubating for 15 min at 20 ºC. Previous experiments demonstrated that longer incubation times did not modify *E. coli* heat

resistance (data not shown). All determinations were performed at least three times on independent working days.

2.5 Incubation of treated samples and survival counting

Tryptone Soy Agar (Biolife) supplemented with 0.6% of yeast extract (TSAYE) was used as recovery medium. Samples of 0.1 and 1 mL were pour-plated in TSAYE and then incubated for 24 h at 35 ºC. Longer incubation times did not change the profile of survival curves. After incubation, colony forming units (CFU) were counted with an improved Image Analyzer Automatic Colony Counter (Protos, Synoptics, Cambridge, UK), as described elsewhere (Condón et al., 1987).

2.6 Resistance parameters

Survival curves to UV-C treatments were obtained by plotting the logarithm of the survival fraction *versus* applied doses, expressed in J/mL, and to heat *versus* treatment times in min.To fit survival curves and to calculate resistance parameters the Geeraerd and Van Impe inactivation model-fitting tool (GInaFiT) was used (Geeraerd et al., 2005). As survival curves obtained in this investigation did not show tails but rather shoulders, the log linear regression plus shoulder model (Geeraerd et al., 2000) was used with the equation:

$$N(d) = N(0).e^{-Kmax.Sl.}\left(\frac{e^{Kmax.Sl}}{1+\left(e^{-Kmax.Sl}-1\right)e^{Kmax.d}}\right) \quad (1)$$

Where *N(d)* represents the number of survivors, *N(0)* the initial count, *d* the applied dose for UV and time for heat treatments, *Sl* the shoulder leght and K_{max} the first order inactivation constant.

This model describes the survival curves through two parameters: the shoulder length (*Sl*) or dose before the exponential inactivation begins and the inactivation rate (K_{max}),

220 defined as the slope of the exponential portion of the survival curve. Therefore, the
221 traditional decimal reduction time value (D) can be calculated from the K_{max} parameter
222 by the equation: $D = 2.303/K_{max.}$ Thermal death time curves were obtained by plotting
223 the Log_{10} 4D values *vs* treatment temperatures, and z values(ºC increase to 4D values
224 decrease ten times) were calculated from the slope of the regression line. Statistical
225 analyses (ANOVA and Student's t-tests) were carried out with the GraphPad PRISM®
226 software (GraphPad Software, Inc., San Diego, Calif., U.S.A.). Error bars in figures
227 correspond to the mean standard deviation.

228 **2.7 Analytical measurements**

229 *2.7.1 Absorption coefficient, turbidity, pH, and ºBrix*

230 Absorbance of orange juice was measured at 254 nm using a Unicam UV500
231 spectrophotometer (Unicam Limited, Cambridge, UK). Sample solutions were diluted
232 and evaluated using quartz cuvettes (Hellma, Müllheim, Germany) with path lengths of
233 1, 2, and 10 mm. The absorption coefficient of the sample solution was determined by
234 the slope of the absorbance *vs*. path length correcting the dilution factor.
235 Turbidity was measured using a HI 83749 nephelometer (Hanna Instrument, Szeged,
236 Hungary). The instrument automatically provides the nephelometric turbidity units
237 (NTU) from lectures of scattered (90º) and transmitted light (180º) detectors with a
238 sample of 10 mL. Measurements were carried out following manufacturer's
239 instructions.
240 The pH measurements of orange juice (20 ± 1 °C) were performed with a pHmeter
241 (Basic 20 pH meter; Crison Instrument, Barcelona, Spain) with a glass electrode
242 (Crison) and calibrated before measurement at pH 4 and 7.
243 The ºBrix of centrifuged samples of orange juice (10 min at 360*g) was measured by a
244 digital hand-held refractometer at 20 ± 1 °C (PR-101, Atago, Tokyo, Japan).

245

246 *2.7.2 Color measurement*

247 Color measurements were carried out as described by Martínez Verdú (2001). For
248 image acquisition, an HP G 4010 Scanjet scanner was used and the images were
249 processed using Matrox Inspector 8.0® software (Stuttgart, Germany). Color calibration
250 was carried out with the HP scanner/Matrox combination, using the 300 Natural Color
251 System (NCS), which make up the Spanish Color Norm (UNE 48-103-94), and CIE
252 1976 (*L**, *a**, *b**) color space (CIELAB) was performed. The redness (*a**), yellowness
253 (*b**) and lightness (*L**) were measured in triplicate. Color difference (*ΔE*) was
254 calculated from *L**, *a**, *b** parameters, using Hunter-Scotfield's equation:

255 $$\Delta E_{ab}=\sqrt[2]{(\Delta L)^2+(\Delta a)^2+(\Delta b)^2} \quad (2)$$

256 Where $a=a-a_0$, $b=b-b_0$ and $L=L-L_0$. The subscript '$_0$' indicates initial color, and the y-
257 intercept of linear regression models was taken for this number. Depending on the value
258 of *ΔE* the development in color difference with treatment could be estimated as not
259 noticeable (0-0.5), slightly noticeable (0.5-1.5), noticeable (1.5-3.0), well visible (3.0-
260 6.0), and greatly different (6.0-12.0) (Walkling–Ribeiro et al.; 2009)

261

262 *2.7.3 Pectinmethylesterase Analysis*

263 The Pectinmethylesterase (*PME*) activity in the orange juice was determined, as
264 described by Kimball (1999), by monitoring the release of acid during pectin hydrolysis
265 as a function of time at pH 7.75 and 20 ± 1 °C. The reaction mixture consisted of 5 mL
266 orange juice sample and 100 mL of a 1% (w/v) citric pectin solution (Sigma-Aldrich,
267 St. Louis, USA) containing 0.15 M NaCl (Panreac, Barcelona, Spain). After mixing
268 components, the pH was quickly adjusted to pH 7.75 and the pH was maintained
269 constant by addition of 0.01 M NaOH. The enzyme activity was related directly to the

amount of NaOH added per minute. One *PME* unit (*PMEU*) was defined as the number of mL 0.01 M NaOH needed per minute to maintain constancy of the initial pH (Eq. 3). The relative *PME* activity was calculated according Eq. 4 (Kimball, 1999). The PME activity of each sample was measured in triplicate.

$$PMEU = \frac{(mL\ NaOH\ 0.01M)}{(5\ mL\ sample \times time[min])} \quad (3)$$

$$Relative\ PME\ activity\ (\%) = \frac{PMEU\ of\ treated\ juice}{PMEU\ of\ untreated} \times 100 \quad (4)$$

2.7.4 Ascorbic Acid Retention

Ascorbic acid (*AA*) content was measured using AOAC's official 2, 6-dichloindophenol titration procedure (AOAC, 1990b). To summarize, 10 mL of orange juice was mixed with 50 mL of 5% acetic acid (Panreac, Barcelona, Spain) as a stabilizing agent and diluted to 100 mL. The mixture was titrated with 2, 6-dichloindophenol solution until a faint pink color appeared and persisted for 15 s. The *AA* content of the samples were calculated by interpolation in a calibration curve previously obtained with several solutions of pure *AA* (AnalaR Normapur, Leuven, Belgium) in 5% acetic acid solution. *AA* retention of treated samples was calculated using the equation:

$$Relative\ AA\ (\%) = \frac{(AA\ treated\ sample)}{(AA\ untreated\ sample)} \times 100 \quad (5)$$

2.7.5 Acidity determination

The acidity (*A%*) of treated and untreated orange juice was determined following the official method described by AOAC (1990a). This is a titration method that use phenolphthalein as indicator. In brief, two mL of orange juice were titrated with 0.1 N NaOH solution until the point when the indicator changed from colorless to pink and

the change persisted for 15 s. Results were expressed as citric acid concentration in 100 mL of orange juice, so that 1 mL 0.1 N NaOH is equivalent to 0.0064 g citric acid.

$$A\% = \frac{mL\ NaOH \times 0.0064 \times 100\ mL\ orange\ juice}{2\ mL\ sample} \quad (6)$$

All analytical assays were performed in triplicate.

3 Results and discussion

In this investigation, the lethal effect of UV radiation, for different times at several temperatures, on *E. coli* STCC 4201 suspended in sterilized commercial orange juice was determined. For comparison purposes, heat resistance of this strain was also performed in the same media. Survival curves of the UV-mild temperatures combined process (UV-H) were fitted with Geeraerd et al.'s model to estimate UV resistance parameters. From data obtained with the model the process parameters for the pasteurization of orange juice was optimized. The effect of this treatment on a pool of five strains of *E. coli* as well as on the content of *AA*, *PME* activity, and other physicochemical characteristics were evaluated in fresh squeezed orange juice.

3.1. UV and heat resistance of *E. coli* in orange juice

The *E. coli* strain STCC 4201 was used to evaluate the UV, heat and the combined treatment (UV-H) in sterilized commercial orange juice since it was the most UV resistant strain of the five *E. coli* strains previously studied (Gayán et al., 2011). Survival curves were constructed by drawing the survival fractions *versus* the applied dose. Traditionally UV dose is expressed as the energy supplied per unit area multiplying the irradiance (W/cm^2) by the exposure time. In continuous flow reactors it can also be expressed in volume units (J/mL). The latter approach is useful to directly compare the energetic efficiency of the process with other technologies (Geveke, 2005;

318 Müller et al., 2011). Figure 1 shows the survival curves of *E. coli* STCC 4201 treated by
319 UV light at different temperatures. As shown in the figure, some survival curves did not
320 follow a logarithmic order of death. Some authors concluded from their work that the
321 number of survivors of UV light treatment may be an exponential function of time
322 (Franz et al., 2009; Oteiza et al., 2010), but many published survival curves to UV light
323 present shoulder and tails (Quintero-Ramos et al., 2004; Unluturk et al., 2010). In this
324 investigation concave downward survival curves were obtained. Also other authors
325 (Hoyer, 1998; Tran and Farid, 2004) have observed no microbial inactivation at low UV
326 doses followed by a normal log-linear relationship at higher UV doses. These shoulders
327 have been related to damage and repair phenomena (Koutchma, 2009). DNA absorbs
328 UV light photons resulting in cross-linking between the neighboring pyrimidine
329 nucleoside bases (thymine and cytosine) in the same DNA strand and thereby causing
330 delay of reproduction or cell death (Bachmann, 1975; Sizer and Balasubramaniam,
331 1999). However, the damage occurring at the DNA level can be repaired up to a level if
332 the recovery conditions are adequate (Liltved and Landfald, 1996). In a previous work
333 (Gayan et al., 2011), it was demonstrated that the shoulder of survival curves of this
334 strain increased when a photoreactivation step was included previous to culture of
335 survivors.
336 There are several mathematical approaches to describe non-linear inactivation kinetics
337 (Mafart et al., 2002; Peleg and Cole, 1998). In this investigation the Log-linear
338 regression plus shoulder model (Geeraerd et al., 2000) was used because it allowed
339 describing independently the shoulders and the Log-linear section of inactivation. The
340 fitting of the survival curves was carried out with the GInaFiT model-fitting tool
341 (Geeraerd et al., 2005) that automatically provides the resistance parameters as well as
342 the treatment time needed to inactivate 99.99% of the initial cell population (*4D*

parameter). The use of the Log-linear plus shoulder model allowed to directly compare results with others previously published (Gayán et al., 2011). Table 1 shows the averages and standard deviations of the UV light resistance parameters calculated with Geeraerd et al.'s model for *E. coli* STCC 4201 suspended in sterilized orange juice and treated at different temperatures. Table 1 includes the coefficient of determination (R^2) and the root mean square error (*RMSE*) values from the fitting, the rate of the log phase inactivation (K_{max}), the shoulder length (*Sl*) and, for comparison purposes, the *4D* parameter. As observed in Table 1, shoulder lengths of survival curves decreased as increasing treatment temperature. Some authors had correlated the shoulders with the damage repair mechanisms (Koutchma, 2009). Therefore results would indicate that damage repair capability of UV treated cells would be lower as treatment temperature increases, or that DNA damages are greater at higher temperatures. On the contrary, the inaction rate (K_{max}) increased with temperature. In summary, the overall lethal effect of UV light treatments drastically increased with temperature. Whereas the highest UV dose possible to apply with the equipment (27.10 J/mL) hardly decreases 0.64±0.04 Log_{10} cycles the population of *E. coli* STCC 4201 at 25.0 ºC, the same doses allowed achieving more than 6 Log cycles of inactivation at 55.0 ºC (Figure 1). In other words, a treatment of 13.55 J/mL reached 0.25±0.04, 0.41±0.13, 0.84±0.32, 0.96±0.12, 2.57±0.05, 3.41±0.23 and more than 6 Log_{10} cycles of inactivation at 25.0, 40.0, 50.0, 52.5, 55.0, 57.5, and 60.0 ºC, respectively (Figure 1). There are few data in literature about the microbial UV resistance in orange juice, probably due to its high absorption coefficient. Koutchma et al. (2007) achieved at room temperature 3.1±0.06 Log_{10} cycles of *E. coli* inactivation in a model juice solution with a similar absorption coefficient than orange juice (48 cm^{-1}) after 3 passes (12.38 J/mL) in a UV coiled tube reactor under turbulent conditions. These results are far from the 5 Log_{10} cycles of inactivation

368 required by the Food and Drug Administration (FDA) juice production regulation
369 (Anonymous, 2001).
370 The improvement of UV lethal effect at mild temperatures has been previously shown
371 by Geveke (2008) in liquid egg white. The population of *E. coli* in egg white was
372 reduced by 1.63±0.10 Log_{10} cycles at a temperature of 30 °C, whereas 2.32±0.07 and
373 2.48±0.41 Log_{10} reductions were achieved when the temperature was raised to 40 and
374 50 ºC respectively, with a UV processing energy of 44 J/mL. Ukuku and Geveke (2010)
375 developed a combined treatment of UV light and radio frequency electric field (20-40
376 ºC) for the inactivation of *E. coli* in apple juice, showing that UV light treatment
377 reached 5.8 Log_{10} reductions, while the combined treatment allowed to achieve 6.2
378 Log_{10} reductions. These results are in agreement with those observations, although the
379 direct comparisons of data are risky because the treatment medium and the equipment
380 used strongly determine the results (Koutchma et al., 2004).
381 To differentiate the contribution of heat to the whole lethal effect, the thermal resistance
382 of *E. coli* STCC 4201 in orange juice was also determinate. Survival curves were fitted
383 with the Geeraerd et al.'s model (2000) and heat resistance parameters were calculated.
384 Table 2 shows the averages and standard deviation of the inactivation rate (K_{max}), the
385 shoulder length (*Sl*), and the *4D* parameter. In a further step it was compared the
386 inactivation parameters for UV light and for thermal treatments at different
387 temperatures. Figure 2 shows the relationship between *4D* parameter and the treatment
388 temperature for both treatments. For UV-H treatments, a concave downward profile was
389 obtained which means that the lethality of UV light hardly changed below 40 ºC, and
390 above this threshold quickly decreased with temperature. For heat treatments, an
391 exponential relationship between *4D* values and temperature was found (Log
392 $4D$=0.221T -11.52, R^2=0.995) as expected. From this thermal death time curve, a *z*

393 value of 4.51±0.23 ºC was deduced. Therefore, an increase in temperature of 4.51 ºC
394 was necessary to reduce the *4D* value by ten times. Similarly, Mazzotta et al. (2001)
395 determined an average *z* value of 4.8 ºC for *E. coli* O157:H7 in orange juice.
396
397 **3.2. Lethal effect of the UV-H combined treatments**
398 In order to determine whether lethal effect of the combined UV-H treatments were due
399 to an additive (the lethality of the combined process was the sum of the inactivation
400 rates of heat and UV light treatments acting simultaneously but individually) or to a
401 synergistic effect (the lethality of the combined process was higher than the sum of
402 lethality of individual treatments), survival curves to UV at room temperature, to heat
403 and to the UV-H treatments at the same temperature were compared (Figure 3). As it is
404 shown, while UV at room temperature and thermal inactivation at 50.0 ºC, 52.5 ºC, and
405 55.0 ºC for 3.6 min was negligible (0.05, 0.08, and 0.61 Log_{10} cycles, respectively), the
406 UV-H at the same temperatures, reduced 2.16, 3.01, and more than 6 Log_{10} cycles,
407 respectively. This demonstrated a synergistic effect of both technologies acting
408 simultaneously. Above 55.0 ºC, the lethality of thermal treatments exponentially
409 increased with temperature and differences among survival curves to heat and the
410 combined process tended to disappear. This phenomenon can be clearly observed in
411 Figure 2. From these results, it was concluded that the lethality of the combined process
412 was the result of a synergistic effect which magnitude was thermodependant among
413 50.0-60.0 ºC. Therefore, it was necessary to optimise the treatment temperature to take
414 full advantage of the combined process.
415 For the optimization of the combined treatment, percent synergism for each temperature
416 was calculated comparing the experimental and the theoretical *4D* values, with the
417 equation:

$$\%\ Synergism = \frac{Theorical\ 4D\ value - Experimental\ 4D\ value}{Theorical\ 4D\ value} \times 100 \quad (7)$$

Theoretical *4D* values were calculated, by assuming an additive effect, with the equation proposed by Raso et al. (1998):

$$Theorical\ 4D_{UV-H} = \frac{(4D_H \times 4D_{UV})}{(4D_H + 4D_{UV})} \quad (8)$$

where $4D_H$, and $4D_{UV}$ values were obtained from the fit of the inactivation curves for the thermal and UV light treatments, respectively.

The magnitude of the synergism at different temperatures can be observed in Figure 4. As it is demonstrated by the figure, the synergism increased with temperature up to 55.0 ºC (68.03%) decreasing further away.

3.3. Effect of optimized UV-H treatment in fresh squeezed orange juice

Several authors have reported significant differences in UV resistance between *E. coli* strains in water systems (Sommer et al., 2000), laboratory media (Gayán et al., 2011) and fruit juices (Basaran et al., 2004; Oteiza et al., 2010). Therefore, the use of a single strain for the determination of a specific dose for a given log reduction is risky (Oteiza et al.; 2010). The Scientific Advisory Panel of the Environmental Protection Agency (EPA) specifically recommended the testing of five outbreak-related strains in a cocktail for each pathogen (Anonymous, 1997). Therefore, the lethal effect of UV-H treatment on a cocktail of five *E. coli* strains (*E. coli* STCC 4201, STCC 471, ATCC 27325, ATCC 25922, and O157:H7) inoculated in fresh squeezed orange juice was performed to validate the designed combined treatment. Figure 5 shows the survival curves of the cocktail in fresh squeezed orange juice treated by UV-H at 55.0 ºC. Survival curves of *E. coli* STCC 4201 in commercial orange juice have also been included for comparison purposes. As observed, both curves overlapped each other and no statistically significant differences ($p > 0.05$) were found between both *Sl* (2.4 J/mL) and K_{max} (0.6

443 mL/J) values. Overall results demonstrated that a UV treatment of 23.72 J/mL at 55.0
444 ºC allowed reaching more than 5 Log_{10} cycles of inactivation of the *E. coli* cocktail in
445 fresh squeezed orange juice.
446 The loss of juice quality and nutritional properties during the processing has become an
447 important issue due to the increase consumer´s demand for fresh food products.
448 Therefore the impact of UV-H combined treatment at 55.0 ºC on physico-chemical
449 properties (*pH*, *ºBrix*, *%A*, *ΔE*), *AA* content, and *PME* activity of natural orange juice
450 was evaluated. For this purpose, measurements were carried out in untreated samples as
451 controls and thermal and UV-H treated fresh squeezed orange juice. Thermal treatments
452 were performed in the same installation tempered at 55.0 ºC with off UV lamps. Results
453 are included in Table 3.
454 Statistical analysis of the results demonstrated that there were no significant differences
455 ($p > 0.05$) between *pH*, *ºBrix*, and *%A* values of the three samples. The total color
456 differences (*ΔE*) of treated samples with the control were 0.23 and 0.07, which were
457 considered to be "not noticeable" changes according to Walkling–Ribeiro et al. (2009).
458 Regarding *AA* loss, the results showed that *AA* was degraded 16.45±0.77% with the UV-
459 H treatment. This loss is believed to be mainly due to UV light because no *AA*
460 destruction was reported in the heat treatment (Table 3). Furthermore the effect of air
461 oxidation of *AA* was measured passing orange juice through the installation at 25.0 ºC
462 with off UV light lamps and it was observed that the *AA* destruction due to air oxidation
463 was negligible (data not shown). UV light is known to generate free radicals through a
464 wide variety of photochemical reactions, which can damage vitamins (Koutchma,
465 2009a). Overall, the percentage of degradation by the combined process was similar to
466 those observed by other authors after UV light at room temperature treatments
467 (Torkamani and Niakousari, 2011; Tran and Farid, 2004).

468 The percentage of *PME* inactivation by the combined and the thermal treatment were
469 63.96±5.07% and 47.84±9.15%, respectively. These values suggested that enzyme
470 inactivation was mainly due to heat. There are few studies about the effect of UV
471 radiation on *PME* enzyme. Tran et al. (2004) obtained an inactivation of 5% when juice
472 was treated with 73.8 mJ/cm^2, and Torkamani and Niakousari (2011) an inactivation of
473 the 8% with the same UV dose. The loss of *PME* activity by the proposed UV-H
474 process was lower than if reached by the traditional thermal sterilization processes (90
475 ºC for 60 sec), that usually pursue the 99.9% loss of *PME* activity (Eagerman, 1976),
476 but it is similar to industrial conditions for heat pasteurization of premium juices (70 ºC
477 for 2 sec) which reduced by 70 % the enzyme activity (Tran and Farid, 2004).
478 Timmermans et al. (2011) demonstrated that thermal treatments at 72 ºC for 20 sec,
479 ultrahigh pressure treatments at 600 MPa for 1 min and high electric field pulses of 76
480 J/mL at 23 kV/cm reached *PME* inactivation levels in freshly squeezed orange juice of
481 85%, 92% and 34% respectively. Therefore the combined process of UV light at mild
482 temperatures could be a realistic alternative to traditional thermal and proposed non-
483 thermal methods for pasteurization of orange juice.
484

485 **4. Conclusions**

486 UV light is a promising technology for the pasteurization of liquid foods. However, the
487 high absorption coefficient of some of them can impair its industrial application. The
488 combination of UV light and mild temperatures synergistically increases the efficacy of
489 the treatment for the inactivation of *E. coli* in orange juice. A UV treatment of 27.10
490 J/mL at 55.0 ºC allowed reaching more than five Log_{10} reductions of a cocktail of five
491 strains of *E. coli*. The treatment does not change the physico-chemical properties or the

juice colour. The loss of ascorbic acid was similar to juice UV treated at room temperature (16.45%), but the decrease of PME activity was much higher (63.96%).

Acknowledgements

This study has been carried out with financial support from the Ministerio de Ciencia e Innovación, EU-FEDER (CIT020000-2009-40) and the Departamento de Ciencia, Tecnología y Universidad del Gobierno de Aragón. E. G. and M. J. S. gratefully acknowledge the financial support for their doctoral studies from the Ministerio de Educación y Ciencia.

References

Anonymous. 1997. Final Report. A Set of Scientific Issues Being Considered by the Agency in Connection with the Efficacy Testing Issues Concerning Public Health Antimicrobial Issues. *U.S. Environmental Protection Agency.* URL: http://www.epa.gov/scipoly/sap/meetings/1997/june/antimicr.htm (Accessed 28 January 2012).

Anonymous. 2001. Hazard Analysis and critical control points (HACCP): Final rule. Federal Register 66. *U.S. Food and Drug Administration,* Washington, DC.

AOAC 1990a. Acidity (as Acid Citric). *Official methods of analysis of the Association of Official Analytical Chemists, 15th ed.,* Association of Official Analytical Chemists, Arlington VA. Method 942.15B.

AOAC 1990b. Vitamin C (Ascorbic Acid). *Official methods of analysis of the Association of Official Analytical Chemists, 15th ed.,* Association of Official Analytical Chemists, Arlington VA. Method 967.21, 1058-1059.

516 Bachmann, R. 1975. Sterilization by intense ultraviolet radiation. *The Brown Boveri*
517 *Review,* 62, 206-209.
518 Basaran, N., Quintero-Ramos, A., Moake, M. M., Churey, J. J. & Worobo, R. W. 2004.
519 Influence of apple cultivars on inactivation of different strains of a *Escherichia*
520 *coli* O157 : H7 in apple cider by UV irradiation. *Applied and Environmental*
521 *Microbiology,* 70(10), 6061-6065.
522 Bintsis, T., Litopoulou-Tzanetaki, E. & Robinson, R. K. 2000. Existing and potential
523 applications of ultraviolet light in the food industry - a critical review. *Journal of*
524 *the Science of Food and Agriculture,* 80(6), 637-645.
525 Condón, S., Oria, R. & Trepat, F. J. S. 1987. Heat resistance of microorganisms: An
526 improved method for survival counting. *Journal of Microbiological Methods,*
527 7(1), 37-44.
528 Condón, S., Arrizubieta, M. J. & Sala, F. J. 1993. Microbial heat resistance
529 determinations by the multipoint system with the thermoresistometer TR-SC
530 Improvement of this methodology. *Journal of Microbiological Methods,* 18(4),
531 357-366.
532 Chapman, P. A., Wright, D. J., Norman, P., Fox, J. & Crick, E. 1993. Cattle as a
533 possible source of verocytotoxin-producing *Escherichia coli* O157 infecction in
534 man. *Epidemiology and Infection,* 111(3), 439-447.
535 Eagerman, B. A., Rouse, A. H. 1976. Heat inactivation temperature–time relationships
536 for pectinesterase inactivation in citrus juices. *Journal of Food Science,* 41(6),
537 1396-1397.
538 Franz, C. M. A. P., Specht, I., Cho, G. S., Graef, V. & Stahl, M. R. 2009. UV-C-
539 inactivation of microorganisms in naturally cloudy apple juice using novel

inactivation equipment based on Dean vortex technology. *Food Control,* 20(12), 1103-1107.

Gachovska, T. K., Kumar, S. Thippareddi, H., Subbiah, J., Williams, F. 2008. Ultraviolet and Pulsed Electric Field Treatments Have Additive Effect on Inactivation of *E. coli* in Apple Juice. *Journal of Food Science,* 73(9), 1750-3841.

Gayán, E., Monfort, S., Álvarez, I. & Condón, S. 2011. UV-C inactivation of *Escherichia coli* at different temperatures. *Innovative Food Science & Emerging Technologies,* 12(4), 531-541.

Geeraerd, A. H., Herremans, C. H. & Van Impe, J. F. 2000. Structural model requirements to describe microbial inactivation during a mild heat treatment. *International Journal of Food Microbiology,* 59(3), 185-209.

Geeraerd, A. H., Valdramidis, V. P. & Van Impe, J. F. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *International Journal of Food Microbiology,* 102(1), 95-105.

Geveke, D. J. 2005. UV inactivation of bacteria in apple cider. *Journal of Food Protection,* 68(8), 1739-1742.

Geveke, D. J. 2008. UV Inactivation of E-coli in Liquid Egg White. *Food and Bioprocess Technology,* 1(2), 201-206.

Guerrero-Beltrán, J. A., Barbosa-Cánovas, G. V. 2004. Advantages and Limitations on Processing Foods by UV Light. *Food Science and Technology International,* 10(3), 137-147.

Hoyer, O. 1998. Testing performance and monitoring of UV systemsfor drinking water disinfection. *Water supply,* 16(1-2), 424-429.

Kimball, D. D. 1999. *Procesado de citricos.* Acribia, Zaragoza, Spain.

565 Koutchma, T., Keller, S., Chirtel, S. & Parisi, B. 2004. Ultraviolet disinfection of juice
566 products in laminar and turbulent flow reactors. *Innovative Food Science &*
567 *Emerging Technologies,* 5(2), 179-189.
568 Koutchma, T., Paris, B. & Patazca, E. 2007. Validation of UV coiled tube reactor for
569 fresh juices. *Journal of Environmental Engineering and Science,* 6(3), 319-328.
570 Koutchma, T., Forney, L.J., Moraru, C.L. 2009. *Ultraviolet Light In Food Technology:*
571 *Principles and Application.* CRC Press, Boca Raton, New York.
572 Liltved, H. & Cripps, S. J. 1999. Removal of particle-associated bacteria by prefiltration
573 and ultraviolet irradiation. *Aquaculture Research,* 30(6), 445-450.
574 Liltved, H. & Landfald, B. 1996. Influence of liquid holding recovery and
575 photoreactivation on survival of ultraviolet-irradiated fish pathogenic bacteria.
576 *Water Research,* 30(5), 1109-1114.
577 Mafart, P., Couvert, O., Gaillard, S. & Leguerinel, I. 2002. On calculating sterility in
578 thermal preservation methods: application of the Weibull frequency distribution
579 model. *International Journal of Food Microbiology,* 72(1-2), 107-113.
580 Maktabi, S., Watson, I. & Parton, R. 2011. Synergistic effect of UV, laser and
581 microwave radiation or conventional heating on *E. coli* and on some spoilage
582 and pathogenic bacteria. *Innovative Food Science & Emerging Technologies,*
583 12(2), 129-134.
584 Martínez Verdú, F. 2001. Chromatic subjects about image capture (II). *Optica pura y*
585 *aplicada,* 34, 1-16.
586 Mazzotta, A. S. 2001. Thermal inactivation of stationary-phase and acid-adapted
587 *Escherichia coli* O157 : H7, *Salmonella*, and *Listeria monocytogenes* in juices.
588 *Journal of Food Protection,* 64(3), 315-320.

589 Müller, A., Stahl, M. R., Graef, V., Franz, C. M. A. P. & Huch, M. 2011. UV-C
590 treatment of juices to inactivate microorganisms using Dean vortex technology.
591 *Journal of Food Engineering,* 107(2), 268-275.
592 Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D. A., Morgan, D. J. & Lyng, J. G.
593 2008. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy
594 for the preservation of fresh apple Juice. *Journal of Food Engineering,* 85(1),
595 141-146.
596 Oteiza, J. M., Giannuzzi, L. & Zaritzky, N. 2010. Ultraviolet Treatment of Orange Juice
597 to Inactivate *E. coli* O157:H7 as Affected by Native Microflora. *Food and*
598 *Bioprocess Technology,* 3(4), 603-614.
599 Peleg, M. & Cole, M. B. 1998. Reinterpretation of microbial survival curves. *Critical*
600 *Reviews in Food Science and Nutrition,* 38(5), 353-380.
601 Quintero-Ramos, A., Churey, J. J., Hartman, P., Barnard, J. & Worobo, R. W. 2004.
602 Modeling of *Escherichia coli* inactivation by UV irradiation at different pH
603 values in apple cider. *Journal of Food Protection,* 67(6), 1153-1156.
604 Raso, J. & Barbosa-Cánovas, G. V. 2003. Nonthermal preservation of foods using
605 combined processing techniques. *Critical Reviews in Food Science and*
606 *Nutrition,* 43(3), 265-285.
607 Raso, J., Pagán, R., Condón, S., Sala, F. J. 1998. Influence of temperature and pressure
608 on the lethality of ultrasound. *Applied and Environmental Microbiology,* 64(2),
609 465-471.
610 Sizer, C. E. & Balasubramaniam, V. M. 1999. New intervention processes for
611 minimally processed juices. *Food Technology,* 53(10), 64-67.

612 Sommer, R., Lhotsky, M., Haider, T. & Cabaj, A. 2000. UV inactivation, liquid-holding
613 recovery, and photoreactivation of *Escherichia coli* O157 and other pathogenic
614 Escherichia coli strains in water. *Journal of Food Protection,* 63(8), 1015-1020.
615 Timmermans, R. A. H., Mastwijk, H. C., Knol, J. J., Quataert, M. C. J., Vervoort, L.,
616 Der Plancken, I. V., Hendrickx, M. E. & Matser, A. M. 2011. Comparing
617 equivalent thermal, high pressure and pulsed electric field processes for mild
618 pasteurization of orange juice. Part I: Impact on overall quality attributes.
619 *Innovative Food Science & Emerging Technologies,* 12(3), 235-243.
620 Torkamani, A. E., Niakousari, M. 2011. Impact of UV-C light on orange juice quality
621 and shelf life. *Food Research Journal,* 18(4), 1265-1268.
622 Tran, M. T. T. & Farid, M. 2004. Ultraviolet treatment of orange juice. *Innovative Food*
623 *Science & Emerging Technologies,* 5(4), 495-502.
624 Ukuku, D. O. & Geveke, D. J. 2010. A combined treatment of UV-light and radio
625 frequency electric field for the inactivation of *Escherichia coli* K-12 in apple
626 juice. *International Journal of Food Microbiology,* 138(1-2), 50-55.
627 Unluturk, S., Atilgan, M. R., Baysal A. H., Unluturk, M. S. (2010). Modeling
628 inactivation kinetics of liquid egg white exposed to UV-C irradiation.
629 *International Journal of Food Microbiology*, 142(3), 341-347.
630 Walkling-Ribeiro, M., Noci, F., Cronin, D. A., Riener, J., Lyng, J. G. & Morgan, D. J.
631 2008. Reduction of *Staphylococcus aureus* and quality changes in apple juice
632 processed by ultraviolet irradiation, pre-heating and pulsed electric fields.
633 *Journal of Food Engineering,* 89(3), 267-273.
634 Walkling-Ribeiro, M., Noci, F., Riener, J., Cronin, D. A., Lyng, J. G. & Morgan, D. J.
635 2009. The Impact of Thermosonication and Pulsed Electric Fields on

Staphylococcus aureus Inactivation and Selected Quality Parameters in Orange Juice. *Food and Bioprocess Technology,* 2(4), 422-430.

Figure 1

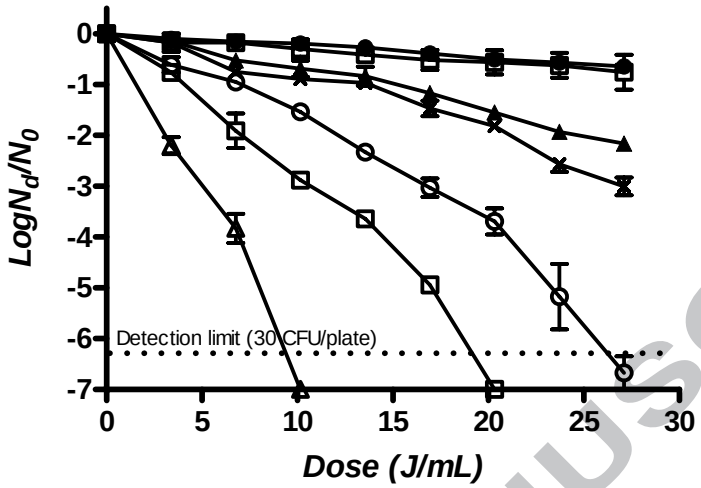

Figure 2

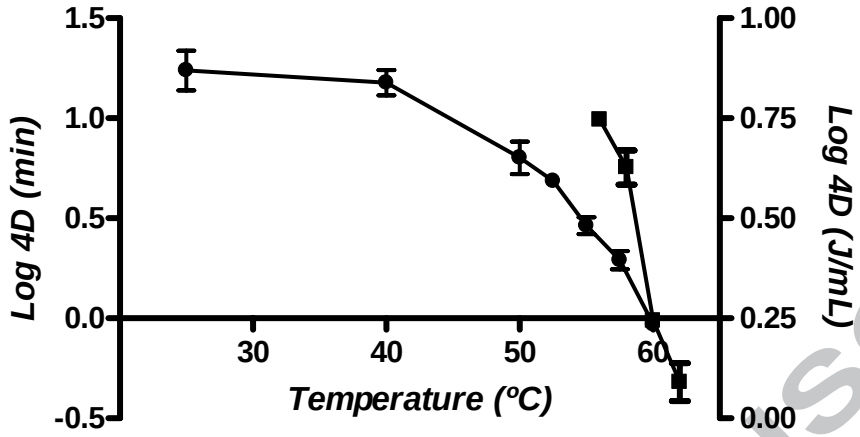

Figure 3

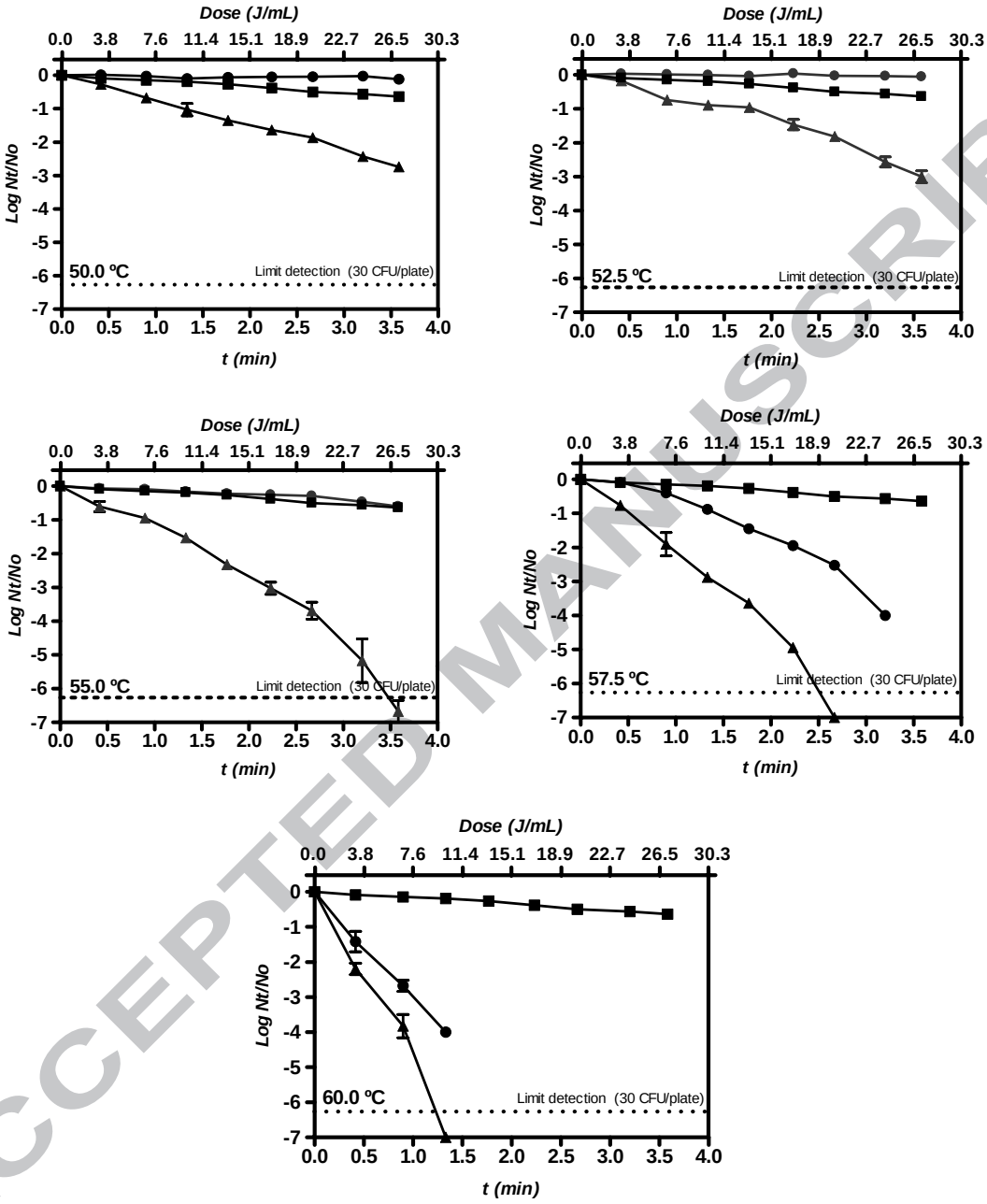

Figure 4

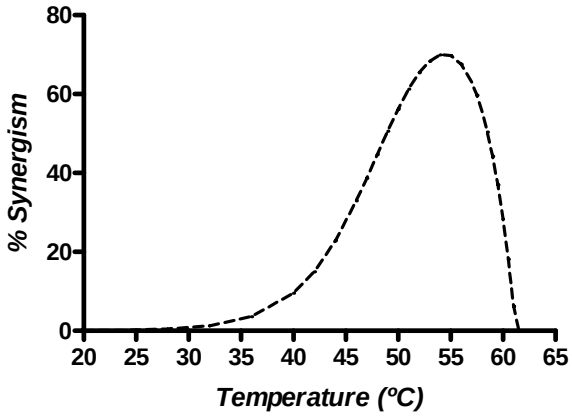

Figure 5

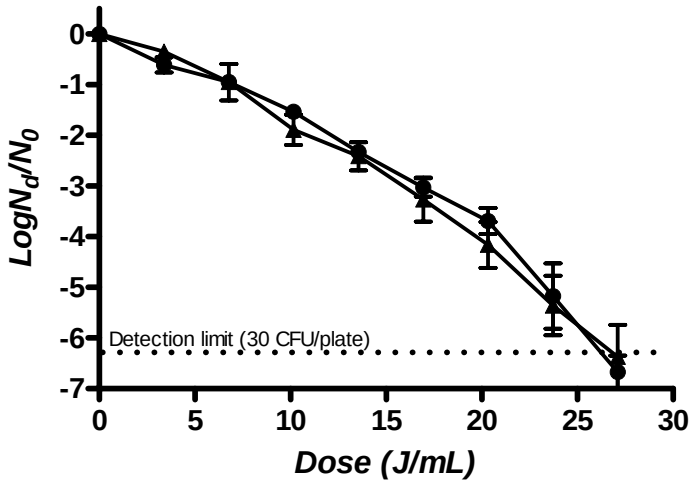

Figure captions

Figure 1. Survival curves of *E. coli* STCC 4201 in commercial orange juice treated by UV light at different temperatures: 25.0 ºC (●), 40.0 ºC (■), 50.0 ºC (▲), 52.5 ºC (×), 55.0 ºC (○), 57.5 ºC (□) and 60.0 ºC (Δ).

Figure 2. Relationship between temperature and *4D* parameter in commercial orange juice for *E. coli* STCC 4201 inactivation by heat (min) (■) and the combined UV-H treatment (J/mL) (●).

Figure 3. Survival curves in commercial orange juice of *E. coli* STCC 4201 treated by UV light at room temperature (■), heat (●) and the combined UV-H process (▲) at 50.0, 52.5, 55.0, 57.5 and 60.0 ºC.

Figure 4. Synergistic effect of the combined UV-H treatments at different temperatures.

Figure 5. Survival curves of *E. coli* STCC 4201 in commercial orange juice (●) and of a *E. coli* strain cocktail (▲) in natural orange juice treated by UV light at 55.0 ºC.

Table 1. UV resistance parameters (*Sl*, *Kmax* and *4D*) at middle temperatures of *E. coli* STCC 4201 in commercial orange juice.

Temperature (°C)	*Sl* (J/mL)	*Kmax* (mL/J)	Dose for *4D* reductions (J/mL)	R^2	*RMSE*
25.0	8.24 (2.58)	0.07 (0.01)	-	0.991	0.025
40.0	6.92 (1.29)	0.07 (0.03)	-	0.984	0.036
50.0	3.99 (0.60)	0.21 (0.01)	-	0.991	0.082
52.5	3.69 (0.46)	0.30 (0.03)	-	0.975	0.187
55.0	2.39 (0.31)	0.61 (0.12)	16.99 (0.25)	0.980	0.401
57.5	1.79 (0.75)	0.91 (0.08)	12.21 (1.06)	0.989	0.194
60.0	0.48 (0.42)	1.72 (0.19)	6.54 (0.22)	0.974	0.374

Table 2. Heat resistance parameters (*Sl*, *Kmax* and *4D*) of *E. coli* STCC 4201in commercial orange juice at different temperatures.

Temperature (ºC)	*Sl* (min)	*Kmax* (min^{-1})	Dose for *4D* reductions (min)	R^2	*RMSE*
56.0	0.23 (0.13)	0.94 (0.08)	9.63 (0.63)	0.997	0.019
58.0	0.15 (0.30)	1.43 (0.14)	6.51 (0.86)	0.988	0.035
60.0	0.13 (0.10)	10.61(0.84)	0.97 (0.23)	0.978	0.298
62.0	0.04 (0.07)	20.81 (2.12)	0.48 (0.09)	0.971	0.473

Table 3. Physico-chemical properties -pH, ºBrix, acidity (*%A*), and colour (*ΔE*)-, ascorbic acid content (*AA*) and pectin methyl esterase activity (*PMEU*) of untreated (*control*), heat (55.0 ºC) and UV-55.0 ºC (*UV-H*) treated fresh squeezed orange juice.

	pH	***º Brix***	***%A***	***ΔE***	***mg AA/100 mL***	***% AA loss***	***PMEU***	***% PME inactivation***
Control	2.83 (0.04)	10.1 (0.10)	1.074 (0.051)		52.45 (1.79)		0.124 (0.018)	
H	2.83 (0.04)	9.86 (0.11)	1.030 (0.037)	0.23	52.84 (0.68)	0	0.035 (0.006)	47.84 (9.15)
UV-H	2.86 (0.03)	9.83 (0.35)	0.975 (0.018)	0.07	43.81 (1.17)	16.45 (0.77)	0.019 (0.083)	63.96 (5.07)

Our reference: JFOE 7025
Editorial reference: JFOE_JFOODENG-D-12-00330
To be published in: Journal of Food Engineering

Highlights

The lethality of UV on *E. coli* suspended in orange juice increased with temperature.

The maximum synergistic effect of UV-H treatment was found at 55 ºC.

A UV-H treatment of 23.72 J/mL at. 55.0 ºC inactivated more than 5 Log_{10} cycles.

The treatment did not affect the pH, acidity, ºBrix and color of orange juice.

The UV-H decreased 16.45% ascorbic acid content and 81.27% the PME activity.