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Adaptation of O157:H7 and non-O157 *Escherichia coli* strains in orange juice and subsequent resistance to UV-C radiation

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ABSTRACT

This study assessed the acid-adaptation of pathogenic and non-pathogenic strains of *Escherichia coli* in orange juice and the microbial resistance to the subsequent UV-C radiation treatment. Nine Shiga toxin-producing *E. coli* (STEC) and one strain of a non-pathogenic surrogate *E. coli* were used in this study. Each *E. coli* strain was inoculated in orange juice, following pre-exposure during 0, 1, 2, and 3 h at 10 °C. Then, the inoculated juices with the ten different strains separately were exposed to 0 and 2 J/cm^2 of UV-C radiation. The *D* value (i.e., the UV-C dose in J/cm^2 required to cause a one-log reduction in the target microorganism) was calculated. Further, the resistance coefficient [RC; i.e., the ratio between the *D*-values for the control condition (D_{0h}) and each pre-exposure tested time (D_{1h}, D_{2h}, D_{3h})] were determined. The results indicated that the resistance of *E. coli* was influenced by the pre-exposure period in the orange juice, with increased resistance to UV-C observed for periods >2 h. Furthermore, the sensitivity of cells to subsequent UV-C treatment was found to be strain-dependent. The results may allow the development of more reliable UV-C radiation processes for orange juice processing aiming the inactivation of pathogenic *E. coli*.

1. Introduction

The worldwide consumption of orange juice is due to its pleasant flavor and vitamin C, minerals, and antioxidants. It is well known that daily consumption of fruits and their derivatives is directly related to reducing several chronic diseases, such as hypertension, stroke, cardiovascular disease, and cancer (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). Paradoxically, foodborne diseases attributed to the consumption of fruit juices represent a severe issue to public health, affecting mainly the more vulnerable people (Preetha et al., 2021). In the period from 1922 to 2019, foodborne outbreaks caused by the consumption of fruit juices have been documented worldwide, causing the death of more than 6500 people, of which 80% were due to the consumption of orange juice and 20% due to apple juice, both unpasteurized and the *E. coli* O157:H7 and *S.* Typhimurium being among the most recurrent pathogens (Krug et al., 2020).

The composition and the low pH levels could make the orange juice a vehicle for developing a wide range of microorganisms, including pathogens able to survive under acidic conditions as *Escherichia coli* strains (Sospedra, Rubert, Soriano, & Mañes, 2012). The acid adaption and the increased resistance to acid stress have been observed in several microorganisms, including *Listeria monocytogenes, pathogenic E. coli*, and *Salmonella* (Hsin-Yi & Chou, 2001). According to Benjamin and Datta (1995), the pre-incubation of enterohaemorrhagic *E. coli* (EHEC) strains in acidic conditions at 37 °C increased their cellular resistance to acidity allowed the bacterial survival to the acidic stomach conditions and thus causing an infection. This acquired tolerance may depend on the cell growth phase, where the maximum resistance was observed at the stationary phase (Zhao, Doyle, & Besser, 1993). This mechanism of adaptation to acid is regulated by gene expressions, where the *rpoS* gene is responsible for codifying an alternative sigma factor (σS). The σS has been recognized as one of the critical factors to produce cell resistance in the stationary phase and cell resistance under several stress conditions. The σS also controls the genic expression of more than 40 specific genes

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related to responses to heat stress, osmotic, oxidative stress, and by the presence of acid and ethanol (Farewell, Kvint, & Nyström, 1998; Yuk & Marshall, 2003).

Although the pasteurization of orange juice offers adequate levels of reduction of the microbial load, the effects on the organoleptic and nutritional characteristics of the product can make it unacceptable by a range of consumers. Thus, it is desirable to find alternatives to mitigate the changes caused by the heat treatments usually used in the food industry. The application of ultraviolet (UV) radiation has been shown as an unconventional alternative to inactivate microorganisms in natural fruit juices (Koutchma, 2019). Nowadays, the UV-C treatment has been successfully applied in a hurdle approach to decontaminate or inactivate different pathogens or spoilage microorganisms. These technologies have been applied mainly with nisin to *Alicyclobacillus acidoterrestris* spores (Ferreira et al., 2020) or with mild heating against *E. coli* O157:H7 (Pagal & Gabriel, 2020). Besides, it has also been combined with ultrasound for inactivating *E. coli*, *Salmonella* spp., and *L. monocytogenes* (Gabriel, 2012). Oteiza, Peltzer, Gannuzzi, and Zaritzky (2005) showed that the exposure of the fruit juice to UV-C wavelengths between 250 and 260 nm produces a substantial effect on microbial inactivation of *E. coli* O157:H7. According to Koutchma (2019), the wavelength of 253.7 nm is the most efficient in terms of a germicidal effect since photons are absorbed by DNA, affecting its normal function. The UV-C treatment of fruit juices has assured up to 5 logarithmic reductions of pathogenic microorganisms (Murakami, Kenji, Nosaka, & Nosaka, 2006).

Nevertheless, the microbial survival or death due to acid adaptation in orange juice or the correlation with the UV-C treatment is not well documented. In this perspective, this work aims to investigate the acid-adaptation phenomena of pathogenic and non-pathogenic strains of *E. coli* in orange juice and their resistance to the subsequent UV-C radiation treatment.

2. Materials and methods

2.1. Bacterial strains

Ten *E. coli* strains from different origins were used in this study. Nine strains belonged to the Shiga toxin-producing *E. coli* (STEC) group, and one strain was a non-pathogenic surrogate *E. coli*, as shown in Table 1.

2.2. Preparation of inoculum

Strains were kept at −80 °C in tryptic soy broth (TSB, Kasvi, Italy) supplemented with 30% glycerol. Cells were reactivated in 5 ml of TSB and incubated at 37 °C for 20–24 h to obtain the bacterial population in the stationary phase. Reactivated cultures were kept at 4 °C until analysis. Before each test, strains were individually transferred to 10 ml of TSB and incubated at 37 °C for 12 h under 200 rpm agitation in a rotary shaker.

Table 1
Characteristics of *E. coli* strains.

Strain	Origin	Serotype	Sorbitol	*eae*	EHEC-*hly*	*stx*
25922	Collection ATCC[a]	ND	+	−	−	−
EDL933	Hamburguer	O157:H7	−	+	+	1 + 2
33/98	Bovine	O157:H7	−	+	+	2
303/00	Salame	O157:H7	−	+	+	2
547/03	Human	O157:H7	−	+	+	2
749/03	Hamburguer	O157:H7	−	+	+	2
646/03	Human	O103:H2	+	+	+	1
870/02	Human	O113:H21	+	−	+	2
002/02	Human	145:NM	+	+	+	2
CIDCA1	Blood saussage	O26:H11	+	+	+	1

[a] American Type Culture Collection.

2.3. Samples preparation and UV-C radiation

Samples (99 ml) of orange juice, previously pasteurized at 95 °C/15 s (°Brix: 13.10 and pH 3.5 ± 0.2), were aseptically dispensed in sterile bottles. Then, the juices were inoculated with 1 ml of bacterial culture in the stationary phase to obtain 10^7 CFU/mL. After inoculation, the samples were pre-incubated during 0, 1, 2, and 3 h at 10 °C. This temperature was selected to simulate consumption conditions normally observed in Argentina. Once the juice is extracted, it is kept under refrigeration conditions (~10 °C) for some time (up to 3 h) before consumption. Subsequently, 5 ml of each inoculated juices were transferred to sterile Petri dishes and exposed to different doses of UV-C radiation (between 0 and 2 J/cm^2) at 20 °C and under agitation at 220 rpm (Oteiza et al., 2005). In all cases, initial counts (N_0) of *E. coli* strains were performed before UV-C treatment by plating cells in TSA (37 °C, 24 h). In addition, for each UV-C treatment, counts of surviving microorganisms (N) were determined. The results were expressed as log CFU/mL. All experiments were performed in duplicate.

2.4. Microbial inactivation

The *D* value is defined as the UV-C dose (J cm^{-2}) required to cause a one-log reduction in the target population. The *D* value was calculated for each strain and condition studied as the inverse of the linear regressions (k) slopes obtained using the relation between the log (N/N_0) and the UV-C dose. The resistance coefficient (RC) is defined as the ratio between the *D*-values for the control condition (D_{0h}) and each pre-incubation tested time (D_{1h}, D_{2h}, D_{3h}).

2.5. Statistical analysis

All data were analyzed by ANOVA, and the differences were determined using Tukey's test ($P < 0.05$) to compare means. In addition, all values were calculated from assays in duplicate using the software Sigma Plot 10 (Systat Software, Inc.), and the box plot graph was made using the software GraphPad Prism 6 (GraphPad Software, USA).

3. Results

In order to observe the effect of adaptation of *E. coli* strains on orange juice and the effect on their resistance to treatments with UV-C radiation, the inactivation values (Log N/N_0) were determined for each strain studied (Fig. 1). Results showed a correlation between the dose of UV-C radiation applied to cells and the inactivation observed, regardless of the strain, but with different magnitudes. Overall, the pre-exposure period of 2–3 h favored *E. coli* survival after the UV-C treatment independently of the strain analyzed or the UV-C dose. Some *E. coli* strains were sensitive to UV-C treatment, such as 33/98, 749/03, and CIDCA1 (Fig. 1b and i). On the other hand, strain 303/00 showed resistance to UV-C treatment and the pre-exposure period in orange juice (Fig. 1c). Also, the pre-exposure period of 0–1 h in the orange juice seemed to negatively affect the resistance of all strains to UV-C treatment (Fig. 1). This finding may suggest a possible mechanism of acid adaptation of studied *E. coli* strains to the orange juice. This phenomenon was observed less intensely for the reference strain ATCC 25922. However, the 3-h pre-exposure period on the orange juice also favored *E. coli* survival compared to other conditions (Fig. 2).

It is critical to highlight that orange juice has a high content of organic acids, the most important and predominant citric acid. This fact can trigger the induction of pH-dependent acid-adaptive mechanisms in the stationary phase, causing an increased resistance of the strains to UV-C radiations (Haberbeck et al., 2017; Lim & Ha, 2021), which can be observed in Table S1. *D*-values of *E. coli* strains in pasteurized orange juice increased as pre-exposure times increased (Fig. 3). These results

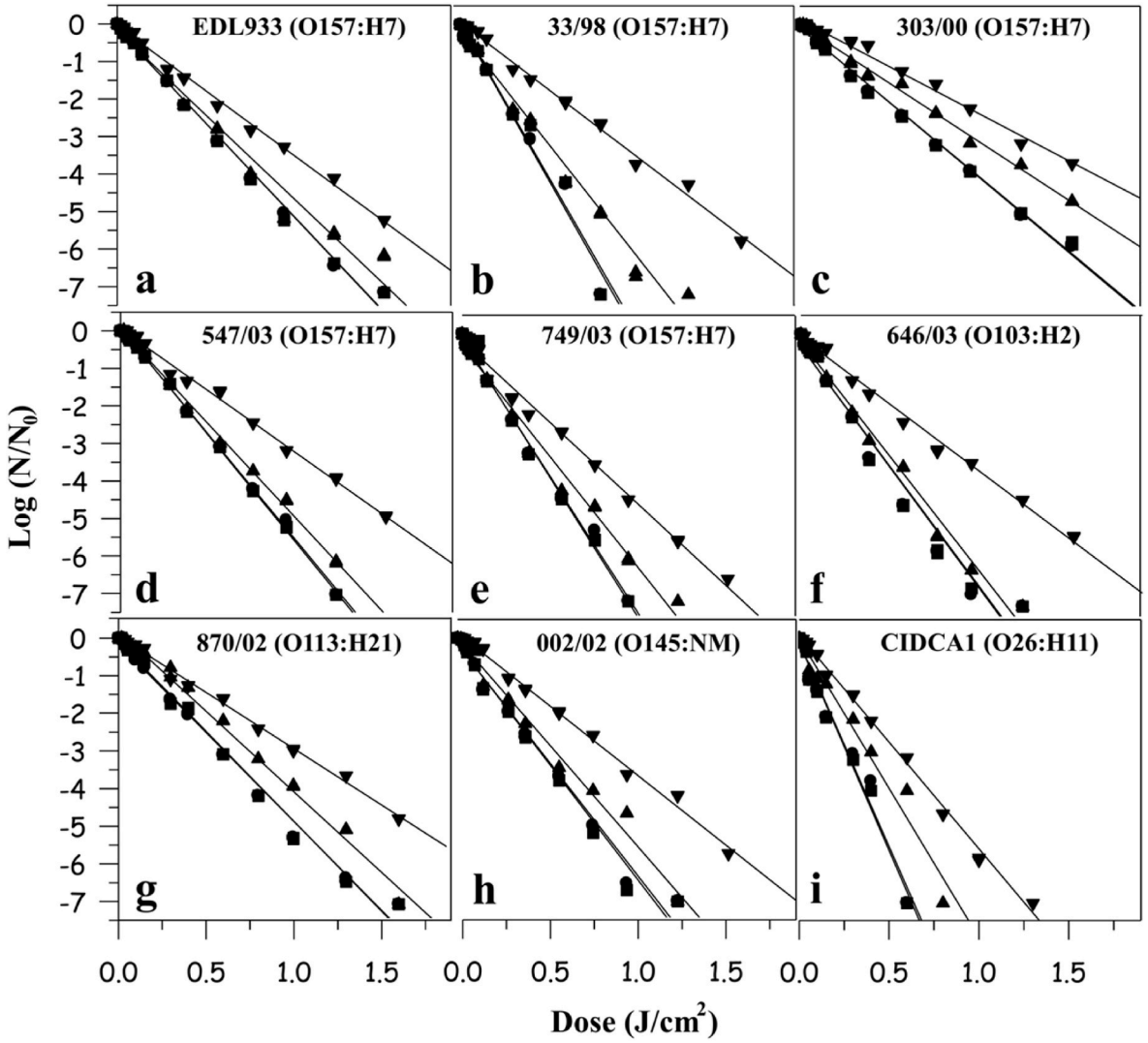

Fig. 1. Inactivation of acid-adapted pathogenic *E. coli* strains (O157:H7 and non-O157:H7). Cells were grown in TSB at 37 °C for 18 h, inoculated in pasteurized squeezed natural orange juice pre-incubated during 0 (•), 1(■), 2 (▲) and 3 h (▼) at 10 °C and subsequently irradiated by UV-C at 254 nm. Strains: (a) EDL933; (b) 33/98; (c) 303/00; (d) 547/03; (e) 749/03; (f) 646/03; (g) 870/02; (h) 002/02; (i) CIDCA1. The TSA medium was used for growth and enumeration.

indicate that a prolonged contact (between 2 and 3 h) of strains with the orange juice acids produces a significant increase of *D*-values for all *E. coli* tested ($p < 0.05$). On the contrary, this effect was not observed when *E. coli* strains were pre-exposed for 1 h.

The resistance coefficient (RC) was calculated for each condition tested to determine the effect of resistance acquired by the strains after the pre-exposure period in orange juice for 1, 2, or 3 h (Table 2). No differences were found between strains pre-exposed during 1 h and the control condition (0 h), with RC values close to 1. On the other hand, RC values varied between 1.05 and 1.39 when *E. coli* strains were pre-exposed for 2 h in orange juice. These values increased from 1.41 to 2.32 when the strains remained pre-exposed for 3 h, indicating an increase in UV-C radiation resistance between 41.4% and 132.2%, respectively. This effect was found to be strain-dependent.

4. Discussion

4.1. Acid adaptation and resistance to UV-C of O157:H7 and non-O157 Escherichia coli are strain-dependent

Adaptation and increased resistance to acid stress have been observed in several microorganisms, including *Listeria monocytogenes*, STEC, and *Salmonella* (Hsin-Yi & Chou, 2001; Topalcengiz, Işık, & Alan, 2019; Usaga, Worobo, & Padilla-Zakour, 2014). The increase in survival of an *E. coli* O157:H7 strain was observed after a 90 min incubation period in the pH range from 4.0 to 5.5, with a maximum adaptation effect induced at pH 5.0. (Koutsoumanis & Sofos, 2004). This phenomenon of acid adaptation has already been documented by Datta and Benjamin (1999), they observed that the low cell density ($<10^7$ CFU/mL) of *E. coli* O157:H7 strains increased their resistance to acid treatment (pH 2.5 for 7 h) by 1000 ×, this effect was partly attributed to the sigma factor *rpoS*, which is involved in the induction of stress proteins in the stationary phase. Bergholz and Whittam (2007) studied the effect of acidity on STEC O157:H7, O26:H11, and O111:H8 strains in a model stomach system and showed a better survival of cells adapted to low pH before exposure to gastric acid, compared to untreated cells.

There are different methodologies to induce acid-enhanced responses, including acid shock and acid adaptation. The acid-shock term is used for cells that have been exposed to a pH gradient, e.g., abruptly from high to low pH, whereas acid-adapted cells are gradually exposed to changes in the pH of the environment (Park, Kim, & Kim, 2017; Usaga et al., 2014). There are several mechanisms involved in the tolerance of pathogens such as *Salmonella* spp. and *E. coli* O157:H7 and non-O157 to acid adaptation: the homeostatic response; the acid tolerance response

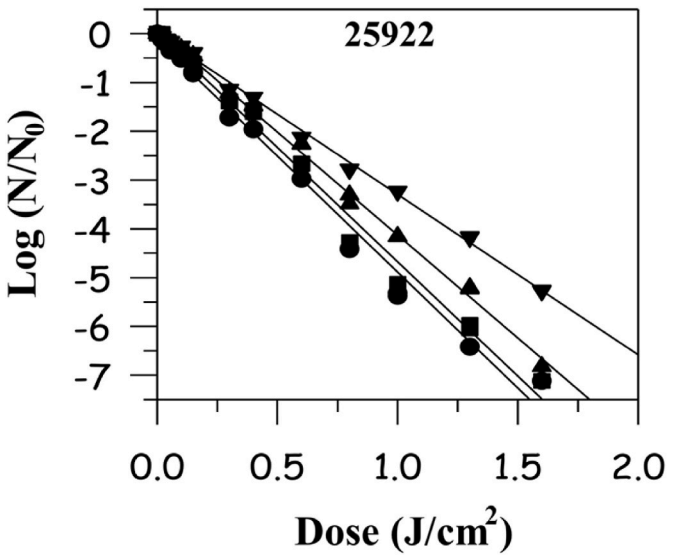

Fig. 2. Inactivation of acid-adapted non-pathogenic *E. coli* strain ATCC 25922. Cells were grown in TSB at 37 °C for 18 h, inoculated in pasteurized squeezed natural orange juice, and pre-incubated during 0 (•), 1(■), 2 (▲) and 3 h (▼) at 10 °C and subsequently irradiated by UV-C at 254 nm. TSA medium was used for the growth and enumeration.

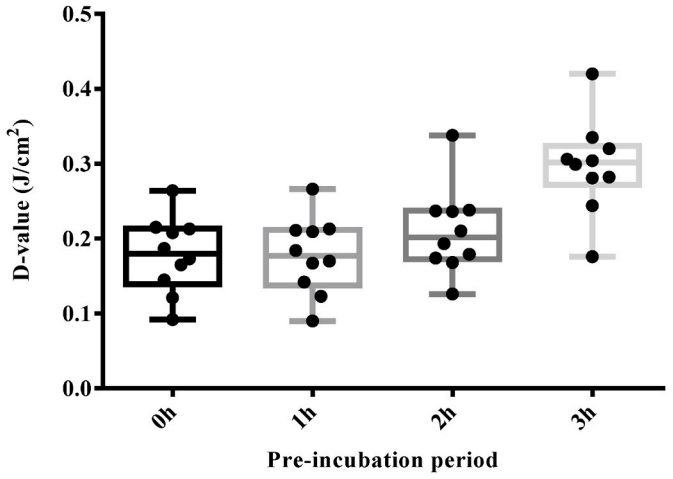

Fig. 3. Box plot of *D-values* of acid-adapted pathogenic *E. coli* strains (O157:H7 and non-O157:H7) at all pre-incubation (0, 1, 2, and 3 h).

Table 2
Values of the resistance coefficient (RC) of acid-adapted pathogenic (O157:H7 and non-O157:H7) and non-pathogenic *E. coli*.

Strain	Resistance coefficient		
	D_{1h}/D_{0h}	D_{2h}/D_{0h}	D_{3h}/D_{0h}
25922 (ATCC)	0.99	1.10	1.41
EDL933 (O157:H7)	0.99	1.11	1.44
33/98 (O157:H7)	1.02	1.39	2.32
303/00 (O157:H7)	1.00	1.28	1.59
547/03 (O157:H7)	0.98	1.12	1.71
749/03 (O157:H7)	0.98	1.23	1.68
646/03 (O103:H2)	1.01	1.05	1.81
870/02 (O113:H21)	1.00	1.14	1.61
002/02 (O145:NM)	0.98	1.11	1.63
CIDCA1 (O26:H11)	0.98	1.37	1.91

RC is defined as the relation between *D*-values at the control condition (D_{0h}) and the pre-incubation (D_{1h}, D_{2h}, D_{3h}).

(ATR); and the synthesis of acid shock proteins, among others (Đeputienė, Supiedėlis, & Supiedėlienė, 2005; Foster, 1991; Heyde & Portalier, 1990; Kanjee & Houry, 2013; Keerthirathne, Ross, Fallowfield, & Whiley, 2016; Ramos-Morales, 2012; Richard & Foster, 2004; Smith, Fratamico, & Gunther, 2014). The pH homeostasis is when a cell maintains a relatively constant intracellular pH over a broad range of external values. This phenomenon was observed in *Salmonella* Typhimurium and *E. coli* (Foster & Hall, 1991).

The ATR is defined as the resistance of cells to low pH when grown at moderately low pH or when cells have been exposed to low pH for a certain period, from 7 to 12 h (Greenacre, Brocklehurst, Waspe, Wilson, & Wilson, 2003). *E. coli* O157:H7 could maintain its vitality in acidic and fermented foods by acquiring resistance to low pH, i.e., ATR. Thus, cells could survive for an extended period in fermented foods such as apple cider (Öztürk & Halkman, 2015). The resistance of *E. coli* O157:H7 varied according to the duration of the adaptation period. Cheng, Yu, and Chou (2003) observed that the resistance of *E. coli* O157:H7 to intense acid stress (pH = 3.0) after exposure to moderate acidic conditions (pH = 5.0) varied according to the adaptation period. Overall, the most pronounced ATR was obtained after 4 h of adaptation.

Several physiological changes occur during treatments with an acid shock, including the synthesis of protective acid stress proteins, which in *E. coli* are regulated by σ^S (*rpoS*) (Usaga et al., 2014). Paul and Hirshfield (2003) observed that the pre-exposure of *E. coli* cells, grown at the log phase, to pH 5.5 and 4.3 induces the synthesis of acid shock proteins responsible for the protection against low pH. Tosun and Gönül (2006) studied the effects of the acid shock and acid adaptation of *E. coli* O157:H7 in TSB, and they reported that acid-shocked cells were exposed to pH 4.5 for 2 h had the highest tolerance to subsequent acid environment.

4.2. Short pre-exposure in orange juice reduces the resistance of E. coli strains to UV-C treatment

Apart from a single stress response, it has been found that exposure to one stress can confer advantages or disadvantages to bacteria to adapt to another stress. These phenomena are known as cross-protection or cross-stress responses (Wang, Buchanan, & Tikekar, 2019). The results shown in the present work agree with those reported by other authors who observed that adaptation to acid causes an increase in resistance to subsequent treatments such as heat (Haberbeck et al., 2017), ultrasound (Patil, Bourke, Kelly, Frías, & Cullen, 2009), non-thermal plasma and UV-C (Ngadi, Smith, & Cayouette, 2003; Wang et al., 2019). However, only limited investigations have been conducted to study the effects of acid adaptation or acid shock resistance associated with emerging non-thermal technologies, especially UV-C radiation. Ngadi et al. (2003) studied the UV-C inactivation of non-acid and acid-adapted cells of *E. coli* O157:H7 in liquid foods. Usaga, Padilla-Zakour, and Worobo (2016) evaluated the UV-C tolerance of acid-shocked and acid-adapted *E. coli* O157H7 in apple juice, and Wang et al. (2019) studied the adaptive response of *E. coli* O157:H7 to UV-C radiation combined with gallic acid prior exposure to sub-lethal stresses (heat and acid) in culture media.

The results of the current study also demonstrated that the pre-incubation period of *E. coli* strains in an acid medium modifies their subsequent resistance to UV-C radiation. Therefore, this behavior must be considered to calculate the UV-C doses (J/cm^2) to achieve a reduction of 5 logarithmic cycles (*5D*) in the *E. coli* population. According to the recommendations of the U.S Food and Drug Administration, in order to guarantee the safety of the fruit juice, and per the HACCP rules, the juice producing plant must guarantee at least 5 log reductions of the target microorganism in question, either by pasteurization or using alternative treatments (Code of Federal Regulations, 2020).

The pathogenic *E. coli* strain 303/00 was the most resistant to the different pre-exposure conditions in orange juice and the UV-C radiation. The radiation dose required to attain a 5 log (*5D*) reduction was 2.10 ± 0.01 J/cm^2 when this strain was pre-exposed in orange juice

during 3 h, whereas the required UV-C dose was 1.32 ± 0.10 J/cm^2 when the irradiation was performed immediately after inoculation in the orange juice (0 h). Therefore, applying this *5D* value to the acid-adapted strain would achieve a reduction of only 3.14 log.

Among the STEC strains, the *E. coli* CIDCA1 (O26:H11) was the most sensitive to the pre-exposure period in orange juice and the UV-C treatments. The UV-C radiation dose necessary to cause a 5-log reduction was 0.88 ± 0.01 J/cm^2 when this strain was subjected to 3 h of pre-exposure period in the orange juice, whereas when the UV-C treatment was carried out immediately after inoculation (0 h), this value was 0.46 ± 0.10 J/cm^2.

The results shown here are of close interest to the orange juice industry, mainly to estimate the UV-C doses necessary to inactivate STEC strains in orange juice. Also, these findings can be used to minimize the time elapsed since the fruit is squeezed until the UV-C treatment is applied, thus avoiding the acid adaptation of pathogenic microorganisms that could be present in orange juice.

5. Conclusions

The prolonged contact between 2 and 3 h of *E. coli* strains in the squeezed orange juice before applying the UV-C treatments caused an increase in microbial's resistance to UV-C radiation. Thus, the control of the time until the product's UV-C light treatment should not exceed 2 h since there is a strong correlation between the ability of *E. coli* strains to adapt to the acid medium and their resistance to UV-C treatment. The sensitivity of cells in orange juice and UV-C treatment was also strain-dependent. These findings may guide the implementation of more efficient control measurements in the juice industry concerning the efficient application of non-thermal methods of microbial inactivation, such as UV-C.

CRediT authorship contribution statement

Juan M. Oteiza: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Magdevis Y.R. Caturla:** Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Leonardo do Prado-Silva:** Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Antonio A. Câmara:** Visualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Patricia A. Barril:** Investigation, Writing – original draft, Writing – review & editing, Validation. **Anderson S. Sant'Ana:** Validation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision. **Leda Giannuzzi:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition. **Noemi Zaritzky:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2022.113107.

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