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# Combined pre-treatments effects on zucchini (*Cucurbita pepo* L.) squash microbial load reduction

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ABSTRACT

Freezing vegetables requires pre-treatments to reduce microbial load and destroy enzymes that impair the frozen product quality. So far blanching has been the most effective pre-treatment, preferred by the food industry, despite its severity: heating up to temperatures close to 100 °C for 1–3 min causes sensory and texture changes in most horticultural products. Alternative blanching treatments, using UV-C radiation combined with milder thermal treatments or with thermosonication, may improve the quality of the final frozen vegetables. Zucchini (*Cucurbita pepo L.*), the vegetable under study, has an availability in fresh restricted to a season, needing therefore to be often frozen to be used throughout the year. In this study, its surface was first inoculated with two vegetable contaminants, *Enterococcus faecalis* and *Deinococcus radiodurans* cells, which are resistant, respectively, to high temperatures and to radiation and then submitted to several blanching treatments, single or combined, and the effect on these microorganisms reduction was evaluated. As single treatments, water blanching (the control treatment, as it is the blanching treatment traditionally used) was applied up to 180 s at temperatures ranging from 65 to 90 °C, and UV-irradiation applied in continuous. As combined pre-treatments, water blanching combined with UV-C (continuous or in pulses), and thermosonication (20 kHz at 50% of power) combined with UV-C pulses were also studied. The continuous UV-C radiation incident irradiance was 11 W/m$^2$ up to 180 s, and the pulses at incident radiance of 67 W/m$^2$, lasting 3.5 s each (35 pulses). Mathematical modeling of bacterial reduction data was carried out using the Bigelow, the Weibull and Weibull modified models, and estimation of their respective kinetic parameters proved that the latter models presented a better fit below 75 °C. The best results proved to be the combination of water blanching at temperatures as low as 85 °C during < 2 min with 25 pulses of UV-C (incident irradiance of 67 W/m$^2$) or thermosonication at 90 °C also combined with UV-C pulses, both resulting in 3 log reductions of both microorganisms under study. These results proved to overcome what industry is requiring so far (a 2 log microbial reduction in 3 min), hence minimizing quality changes of frozen zucchini.

## 1. Introduction

Fresh fruits and vegetables have both important nutritional and economic value. Recently, consumers started revealing an increasing interest on the relationship between their health and the nutritional aspects of food, namely of horticultural products (vitamins content, mineral elements, antioxidants, *etc.*) (Scalzo et al., 2005). Zucchini (*Cucurbita pepo L.*) is a small summer marrow or green squash, with a shape resembling a ridged cucumber. It is usually available in fresh, being consumed raw with skin in salads, or served cooked in soups or other recipes. Due to the advantages of frozen produce (nutritional quality retention and a much longer shelf life, allowing its availability in all seasons), commercial frozen chopped zucchini has become more popular (Gebczynski and Kmiecik, 2007). Industrial production of frozen vegetables involves a thermal pre-treatment named blanching that involves water or steam (Canet, 1989), and uses temperatures ranging from 70 to 100 °C lasting from seconds to a few minutes. It aims to reduce microbial load, inactivate enzymes (Neves et al., 2012) responsible for unwanted sensory changes during frozen storage (Brewer et al., 1995) and, through its washing action, to eliminate off-flavors (possibly formed since harvesting and until processing) and/or to remove residual pesticides (Canet, 1989; Kleinschmidt, 1971; Préstamo et al., 1998; Shams and Thompson, 1987). The severity of this process should be reduced as it imparts pigment modifications, tissue softening

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or nutrient losses, impairing quality (Aguilar et al., 2004; Goodman et al., 2002; Hoover, 1997). Moreover, from an industrial point of view, this process may be extremely energy consuming.

In an attempt to respond to this problem, there is a growing interest on the use of ultraviolet (UV) radiation as a non-thermal process to be used alone or combined with less severe thermal processes in post-harvest storage of fruits and vegetables (Allende and Artés, 2003; Martens and Knorr, 1992). The short wavelength (254 nm) UV-C can be lethal to most microorganisms, probably due to the penetration of radiation on the outer cell membrane, damaging the microorganism's DNA, preventing it to transcript and replicate (Britt, 1996; Tuteja et al., 2001; Tuteja and Tuteja, 2001), and leading eventually to cell death (Bank et al., 1990; Bintsis et al., 2000; Miller et al., 1999). UV-C radiation is a cold and dry process requiring very low maintenance (Morgan, 1990), but with limited applications (surfaces or transparent liquids) due to its low penetrating capacity. It does not produce by-products nor generate chemical residues that could change the sensory characteristics of the final product or deliver residual radioactivity as ionizing radiation (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Due to the wide variety of organisms present on food surfaces, the most critical factor to be controlled, is the required radiation dose (fluence), commonly measured in $J/m^2$. This variable depends on the distance from the UV source to the product and on the exposure time (high fluencies, although greatly reducing the number of microorganisms, may produce discoloration and accelerate the senescence of the product). UV-C irradiation may also be applied as pulses using high voltage electricity discharges for very short periods of time (seconds) after thermal processing (Dunn, 2001; Morris et al., 2007).

Another non-thermal technology nowadays in focus is thermosonication, which is the application of ultrasounds, at high power (typically in the range 10–1000 $W/cm^2$), in water at temperatures in the range of water blanching (Cruz et al., 2013).

*Enterococcus faecalis*, is a regular vegetable contaminant that grows and spoils vegetables, very quickly, at environmental temperatures. Due to its high resistance to freezing or pasteurization temperatures, for long periods of time (Adams and Moss, 1995; Hartman et al., 1992; Perez et al., 1982; Ray, 1996; Temiz, 1998), it can be a good indicator of thermal and non-thermal treatments efficiency. *Deinococcus radiodurans* resists to extreme conditions of life, such as excessive desiccation and exposures to high fluencies of ionizing and ultraviolet radiation, as well as many toxic chemicals (Battista, 1997; ; Minton, 1996; Blasius et al., 2008). This microorganism can be a good indicator to test the efficiency of UV-C irradiated food, when assuming that resistant microorganisms to radiation are present.

This study was intended to develop a milder blanching treatment, when compared to the traditional water blanching, to apply to zucchini, by replacing this treatment totally or just partially, by combining it (at lower temperatures and shorter times if possible) with UV-C radiation combined and/or with thermosonication. Two microorganisms were used to test the efficiency of all the treatments studied, *E. faecalis,* and *D. radiodurans* for the reasons described above.

## 2. Materials and methods

### 2.1. Inoculums preparation

The strains of *E. faecalis* (ATCC 29212) and *D. radiodurans* (ATCC 13939) used in this study were acquired from the American Type Cultures Collection (ATCC) as a lyophilized pellet in a stick device (kwik-stik™). To prepare the inoculum, the procedure used was the same for both microorganisms. Pellets of each strain, were first rehydrated and mixed with the fluid present in the device. Both primary cultures were inoculated by means of a swab in Petri dishes with Plate Count Agar (PCA) (Scharlou, Scharlab S.A., Spain), and incubated at 37 °C during 24 h. Next, a loop of growing cells taken from the inoculated area of each strain, was re-streaked in another PCA plate and incubated for more 24 h. To obtain stationary growing phases, a loop of growing cells taken from the inoculated area of each strain was then, for *E. faecalis* ATCC 29212, submerged in 250 mL of Brain and Heart Infusion (BHI) (Scharlou) at 44 °C during 15 h, and for *D. radiodurans* ATCC 13939 placed in a Nutritive Broth (Scharlou) at 37 °C during 24 h. Next, 10 mL of the initial inoculum was added to 2 L of sterilized water, giving a microbial load of approximately $10^4$ viable microorganisms.

### 2.2. Raw material

Zucchini (*Cucurbita pepo* L.) squashes (15–20 cm length and 5–6 cm diameter) were obtained in a local supermarket, in Faro - Portugal. All vegetables were carefully selected, free of disease symptoms and defects, with uniform size and color. From each vegetable, the edges were first discarded and the obtained cylinders were manually cut lengthwise in four parts with a stainless steel knife hereinafter called quarters.

### 2.3. Chemical disinfection

The zucchini (*Cucurbita pepo* L.) quarters were immersed in a bath containing 2 L of distilled and sterilized water in a 5% solution of sodium hypochlorite, which is recognized as GRAS (generally recognized as safe) (prepared from a commercial brand – W5), with constant mixing during a contact time of 10 min. Afterward, the samples were rinsed in distilled sterile water to remove the chemical residues and then dried on open air.

### 2.4. Sample inoculation

Right after disinfection, zucchini pieces were immersed for 10 min in the single inoculum-broth, prepared as described above, and next were let dry in open air.

### 2.5. UV-C radiation equipment

The source of UV-C radiation was a set of 4 separated and unfiltered 16 W TUV (Philips) germicidal lamps mounted in the top part of a chamber at a distance of 10 cm from the sample holder. This equipment was designed at the Food Engineering Department of University of Algarve, Portugal. In order to maximize the intensity of the incident radiation, a preliminary study with the help of a digital photometer (DO 9721 Delta OHM, Caselle di Selvazzano, Italy) was carried out to find the spot on the plate where this radiation would be maximum. During this study, a ventilation device was installed in the back of the chamber to avoid temperature increase. Right after switching on the device, the radiation intensity reached a peak, followed by a decrease of intensity levelling into a plateau (stabilization) after ≈10 min.

### 2.6. Enumeration

The germicide effect of UV-C radiation was only evaluated on the whole vegetable surface with and without any kind of chemical disinfection.

Ten grams of tissue from the quarters' peel were aseptically removed with a sterile scalpel and pummeled in a stomacher (Seward, Stomacher 400), using 90 mL of Buffered Peptone Water (BPW) (Scharlou) for 3 min. Appropriate decimal dilutions in BPW were performed. All microorganisms were enumerated in duplicate.

#### 2.6.1. E. faecalis ATCC 29212 bacteria

These bacteria were grown at 44 °C for 48 h in Slanetz and Bartley Agar (Scharlou). The colonies presented a wine-red color.

#### 2.6.2. D. radiodurans ATCC 13939 bacteria

These bacteria were incubated in PCA (Scharlou) enriched with 1% of D-Glucose Anhydrous (Merck) at 37 °C during 24 h. The colonies were

white. All the procedure was carried out using a Microflow Security Cabinet (Bioquell, Germany) in order to prevent post-contamination.

### 2.7. Pre-treatments applied to zucchini

In all the pre-treatments studied, zucchini was first inoculated with the microorganism in study (either *E. faecalis* or *D. radiodurans*) according to the procedure described in Section 2.4. As a control, zucchini quarters, disinfected and inoculated, but without any application of pre-treatment, were used in all the experiments described. Inoculation levels were in the range of $3 \times 10^4 \pm 1 \times 10^4$ CFU/mL, considered the level of contamination found in nature. Due to this variation in counts for each inoculation, it was decided that bacteria enumeration should always be evaluated in a relative way. Therefore, in each experiment, the initial microbial count was determined and each count after the treatment (N) was divided by the initial count ($N_0$) in order to obtain relative degradations.

#### 2.7.1. Single pre-treatments

As single pre-treatments, hot water immersion (used as control treatment) and two kinds of UV-C treatments (1. continuous incident irradiance for 1.5 min at 11 W/m$^2$ and 2. discontinuous fluence of radiation for 25 pulses at 67 W/m$^2$ incident irradiance) were carried out. The temperature inside the equipment was monitored with a thermocouple (constantan-type T, 1.2 mm diameter) and mean temperature during the treatments was 25 ± 2 °C. The fluent radiation was monitored by the UV digital photometer (DO 9721 Delta OHM, Caselle di Selvazzano, Italy). Safety goggles and protective gloves were also used. All experiments (single and combined) were run in triplicate.

*2.7.1.1. Continuous fluence UV-C radiation treatments on zucchini.* Prior to use as a continuous source of radiation, the UV-C lamps were allowed to stabilize by waiting 10 min after switching on the lamps until a plateau was reached of 11 W/m$^2$ (Fig. 1*a*). After that, the door was opened, a sample was placed quickly in the sample holder, and the door was shut right away. Irradiation times varied from 15 up to 180 s (fluences ranging from 165 up to 1980 J/m$^2$).

*2.7.1.2. Discontinuous fluence (pulses) of UV-C radiation treatments on zucchini.* The idea of applying pulses came from the observation that the lamps emitted right after being turned on a much higher intensity of radiation (peak) of 67 W/m$^2$ (Fig. 1*a*). Pulses could therefore be produced, by turning the switch on and off every 3.5 s (Fig. 1*b*). Each pulse being equivalent to 3.5 s of irradiation time. The pulses applied varied from 5 pulses (equivalent to 17.5 s, fluence − 1172.5 J/m$^2$) up to 35 pulses (122.5 s, fluence - 8207 J/m$^2$).

*2.7.1.3. Thermal treatment (water blanching).* Zucchini quarters were immersed in a thermostatic water bath (Grant, model W14, Cambridge, England), with 10 L capacity, at six different temperatures in the range of 65 °C to 90 °C. Samples were taken out from the water bath after the required processing times, ranging from 10 to 180 s, and immediately cooled down in an ice water bath. The water bath temperature was monitored with a digital thermometer (Ellab ctd 87) and a thermocouple (type T, 1.2 mm diameter).

#### 2.7.2. Combined UV-C treatments with thermal treatment (blanching)

A combined UV-C radiation/thermal treatment was next studied. As the effect difference of the sequence, UV-C application before blanching irradiation or blanching before UV-C irradiation was unknown, a preliminary experiment was run at temperatures of 60 and 90 °C to test the microbial reduction of *E. faecalis* with both sequences leading to the conclusion that UV-C radiation should be first applied. Hence, contaminated zucchini (*Cucurbita pepo* L.) quarters, were first exposed to a continuous fluence of UV-C radiation or to UV-C pulses, as described above, followed by being subjected to the same thermal treatment described in Section 2.7.1.3. Right before the radiation treatment, three quarters were collected for microorganisms' enumeration to evaluate the initial microbial load.

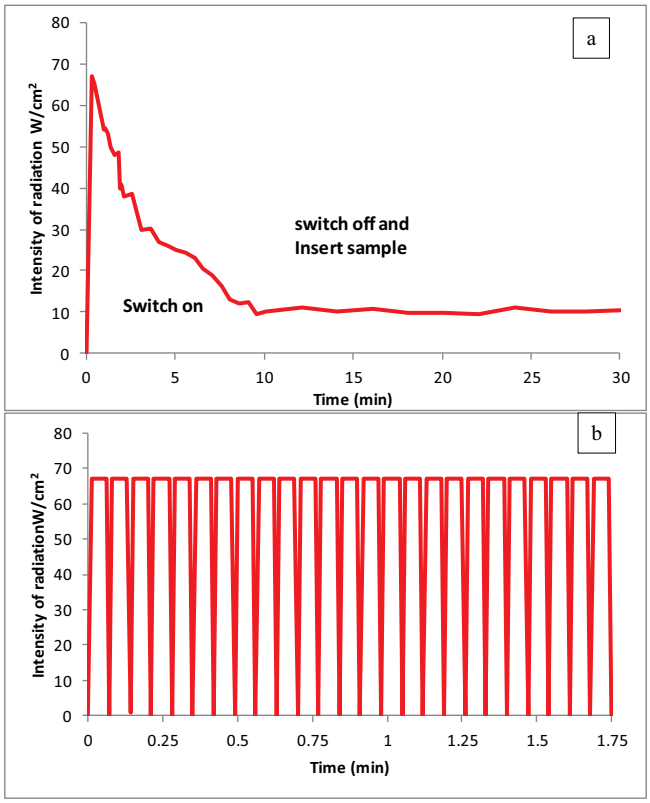


**Fig. 1.** UV-C radiation time profile emitted on the prototype of equipment: *a*) emitted in continuous; *b*) emitted by pulses.

#### 2.7.3. Discontinuous fluence of UV-C radiation combined with thermosonication

A combined UV-C pulsed radiation/with thermosonication applied to zucchini (*Cucurbita pepo* L.) quarters, for the same range of temperatures, was the last treatment applied. The procedure followed was the same as in Section 2.7.3, except for the thermal treatment that was replaced by thermosonication. The water bath where samples were immersed had now coupled an ultrasound horn (Cole Parmer V1A; 13 mm dia) at 20 kHz and an ultrasound generator (Cole-Parmer 4710 Series) radiating 50% of power.

### 2.8. Data analysis

#### 2.8.1. Modeling of the inactivation behavior

Survival curves for both *E, faecalis* and *D. radiodurans* were generated from the experimental data by plotting log $N/N_0$ (where N is the number of colony-forming units CFU/mL at a given time, and $N_0$ the initial number of CFU/mL) *versus* time of blanching. To describe the behavior of the two microorganisms, three mathematical models were attempted, the Bigelow model, the Weibull model and a modified version of the latter, as described below and showed in Table 1.

*2.8.1.1. Bigelow model.* The Bigelow model, being a conventional first-order model, is based on the assumption that the microbial populations, have a homogeneous resistance to heat, being in Eq. (1) $N_0$ the initial and $N$ the final (at t time of exposure) microbial populations in CFU/mL and $N/N_0$ the survivors fraction,

**Table 1**
Models used to explain better the experimental data and kinetic parameters.

| Model | Equation | | References |
|---|---|---|---|
| Bigelow | $\frac{N}{N_0} = 10^{-\left(\frac{t}{D_{(T_{ref})}10^{\left(\frac{T-T_{ref}}{z}\right)}}\right)}$ | (9) | Bigelow, 1921 |
| Weibull-Peleg | $\frac{N}{N_0} = 10^{-(\ln\{(1+\exp[k_0(T-T_c)]\}.t^p)}$ | (10) | Corradini et al., 2005; Fernández et al., 1999; Peleg, 1999; Peleg and Cole, 1998, 2000; Weibull, 1951 |
| Weibull-Mafart | $\frac{N}{N_0} = 10^{-\left(\frac{t}{\delta_{(T_{ref})}10^{\left(\frac{T-T_{ref}}{z}\right)}}\right)^p}$ | (11) | Mafart, 2005; Purich and Allison, 2000 |

$$\log\left(\frac{N}{N_0}\right) = -\frac{t}{D_{(T)}} \quad (1)$$

The temperature dependence of the decimal reduction time $D_{(T)}$ may be described by the Bigelow model (1920) (Eq. (2)) (Purich and Allison, 2000):

$$D_{(T)} = D_{(T_{ref})}10^{\left(\frac{T_{(ref)}-T}{z}\right)} \quad (2)$$

where z represents the temperature increase to produce one decimal reduction in $D_{(T)}$ (°C) and $T_{ref}$ the average of experimental temperatures (K).

*2.8.1.2. Weibull-Peleg model.* The Weibull model (Eq. (3)), on the other hand, considers that the heat resistance of a microbial population may vary, mainly due to its growth phase, being able to model with a good fit the inactivation of microorganisms by several factors, such as heat or radiation (Fernández et al., 1999; Peleg, 1999; Peleg and Cole, 1998, 2000).

$$\log\left(\frac{N}{N_0}\right) = -k_T t^p \quad (3)$$

It introduces a parameter $p$ (the time power), the "shape parameter" related to the survival curve shape and independent from temperature. The value of $p$ indicates whether the surviving microbial cells have the ability to adapt to the applied stress ($p < 1$, curve upper concavity in tail shape), or are in a very injured condition ($p > 1$, downward concavity in shoulder shape). When $p = 1$, a linear behavior is observed, meaning that the system follows the Bigelow model (Eq. (2)) (Albert and Mafart, 2005). $k_{(T)}$ on the other hand is temperature dependent. Regarding this dependence, several models can describe it, being the most well known the Arrhenius model (Eq. (4)), which considers that all processes causing death of microbiota in their vegetative or spore form are defined by a single activation energy *Ea* (J/mol), implying that all present microorganisms have the same resistance to the applied process.

$$k = k_{ref}\exp^{-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \quad (4)$$

where:

*R- is the* Universal gas constant (8.314 J/mol K).

*T* - Temperature of the isothermal experiment (K).

$k_{ref}$ - Value of k at the reference temperature $T_{ref}$.

However, there are other models that take into account different resistances to thermal inactivation, such as the log logistic model (Eq. (5)), recommended for microbial inactivation by several authors (Peleg, 2002; Peleg, 2003; Corradini et al., 2005),

$$k_{(T)} = \ln\{1 + \exp[k_0(T - T_c)]\} \quad (5)$$

where $T_c$ is the critical temperature, and marks the temperature at which destruction starts to accelerate, and $k_o$ is the slope of the linear part of the $k_{(T)}$ curve, at temperatures much higher than $T_c$.

*2.8.1.3. The Weibull-Mafart model.* A modification of the Weibull model, (Mafart et al., 2002) was also tried, where a new parameter was introduced ($\delta$), which represents the time needed for the first decimal reduction cycle on the microbial population to take place, and is related to the constant rate as shown in Eq. (6).

$$\log\left(\frac{N}{N_0}\right) = -\left(\frac{t}{\partial_T}\right)^p \quad (6)$$

Parameter $\delta$ and the constant rate ($k$) are related, as shown in Eq. (7), and depends on temperature in a similar way as parameter $D$ in the Bigelow model (Eq. (8)).

$$\partial_{(T)} = \left(\frac{1}{k_{(T)}^{1/p}}\right) \quad (7)$$

$$\partial_{(T)} = \partial_{(T_{ref})}10^{\left(\frac{T_{(ref)}-T}{z}\right)} \quad (8)$$

To model the experimental data, the models shown in Table 1 were used, where parameters $D_{(T)}$ of Eq. 1, $k_{(T)}$ of Eq. (3) and $\delta_{(T)}$ of Eq. (5) were replaced by the equations that better describe their temperature dependency (Eqs. (2), (4) and (6), respectively). The kinetic parameters were estimated for each model directly from the experimental data, by performing a non-linear regression analysis (Arabshahi and Lund, 1985). The reference temperature used was the average value of the range considered, aiming at improving the parameter estimation.

### 2.8.2. Statistical analysis

To allow the selection of the best model, internal validation was carried out through the precision of the estimated parameters for all models studied, evaluating the confidence intervals at 95%. Also quality of regressions was assessed by the coefficient of determination through the evaluation of the adjusted coefficient of determination ($R^2_{adj.}$), $p$-value, standard deviation and randomness and normality of residuals (Hill and Grieger-Block, 1980). To carry out all the statistical analysis the Statistical Software STATA version 10.0 was used (Statacorp, 2007).

### 2.9. Key resources table

| Resource | Source | Identifier |
|---|---|---|
| Chemical D-Glucose Anhydrous | http://www.merckmillipore.com/PT/en/product/D+Glucose-Anhydrous-CAS-50-99-7-Calbiochem,EMD_BIO-346,351 | D(+)Glucose, Anhydrous - CAS 50–99-7 - Calbiochem |
| kwik | https://www.microbiologics.com/item-type/Product/product-format/KWIK-STIK-2-Pack,KWIK-STIK-6-Pack?order=storedisplayname:-asc&display=list | KWIK-STIK™ lyophilized microorganism pellet, ampoule of hydrating fluid and inoculating swab |
| Sodium hypochlorite | https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-hypochlorite | SODIUM HYPOCHLORITE 7681-52-9 |
| stik | https://www.microbiologics.com/item-type/Product/product-format/KWIK-STIK-2-Pack,KWIK-STIK-6-Pack?order=storedisplayname:-asc&display=list | KWIK-STIK™ lyophilized microorganism pellet, ampoule of hydrating fluid and inoculating swab |

## 3. Results and discussion

### 3.1. Single treatments applied

In Fig. 4, first two columns, *a (E. faecalis)* and *b* (*D. radiodurans*) under WB, the standardized survivor counts ($N/N_0$) are shown for both microorganisms in a 3-log cycle scale, for all the tested temperatures and up to 3 min. At 65 °C, very little effect is observed in both microorganisms reduction. At 70 and 75 °C, by 3 min, the number of survivors of *E. faecalis* reached a non-detectable level, leading to the conclusion that a 3 $\log_{10}$ reduction was achieved by this time and assuming a Bigelow temperature dependence above this temperature it would be expected to obtain the same reduction in shorter processing times. However, at 80 and 85 °C, a deviation from log-linear behavior was observed as some bacteria were detected by 3 min (pointed in red in Fig. 4) contradicting the previous results. As for *D. radiodurans* up to 80 °C less than a 1 $\log_{10}$ reduction was achieved after 3 min, and for higher temperatures only a 2 $\log_{10}$ reduction was achieved by the same time. Again, a deviation from a log-linear behavior is observed, enhanced with an increase in temperature.

Concerning the effect of single UV-C radiation treatments on both microorganisms, when applied continuously (11 J/m$^2$), UV-C irradiation does not cause much effect on *E. faecalis* reduction and even when applied in pulses of 67 J/m$^2$ each, only a 1 $\log_{10}$ reduction was obtained as observed in (Fig. 2 *a*). This result is agreement with the reported 1 $\log_{10}$ reduction on *E. faecalis* ATCC 29212 by Gayán et al., 2014 (66 J/m$^2$ for a 1 $\log_{10}$ reduction). Manzocco et al. (2011) achieved reductions in the range of 2.14–2.32 log CFU/g for enterobacteriacae while cutting melon under UV-C light at different fluences (1.2, 6000 and 12,000 J/m$^2$) up and times (1, 5, and 10 min) proving that shorter periods of PUV exposure are more effective than UV-C. As for *D. radiodurans* (Fig. 2 *b*) a continuous UV-C treatment (11 J/m$^2$) for 90 s did not have any significant effect on this resistant microorganism. The behavior of *D. radiodurans* to radiation is not surprising, as *D. radiodurans* is very resistant to UV-C radiation, the reason why it was used in this study. In the Gayán et al., 2014 study, it was published for this strain under the designation *Micrococcus radiodurans* ATCC 13939, that for a 1 $\log_{10}$ reduction a fluence of 198.6 J/m$^2$ is needed. It is known that the mechanism of microbial inactivation caused by UV-C radiation occurs because of protein structure modifications other than those derived from conventional thermal blanching (Daly et al., 2007; Daly, 2012). Resistance to microbial inactivation is therefore due to its ability to produce protein protection mechanisms such as powerful antioxidants (Minton, 1996; Battista, 1997; Cox and Battista, 2005; Blasius et al., 2008).

The nature and composition of food surface, the degree of attachment to food or association of microorganisms with food and biofilms formation are other possible justifications (Mohamed and Huang, 2007).

### 3.2. Combined treatments applied

When combining UV-C (continuous or pulsed) with blanching treatments, the sequence UV-C irradiation followed by blanching, proved to be significantly more effective in reducing *E. faecalis* than the other way around, at both 60 and 90 °C, (Fig. 3, *a* and *b*), being the effect much more noticeable at the lower temperature. In fact, when WB is applied first, water droplets stay on top of the zucchini skin causing a shield to UV-C radiation to penetration on the area covered by the droplet and a shadow effect.

By observing Fig. 4 again, the application of UV-C in continuous followed by water blanching (UV-C + WB) hardly any improvement is observed when compared to single WB until 80 °C is reached. For higher

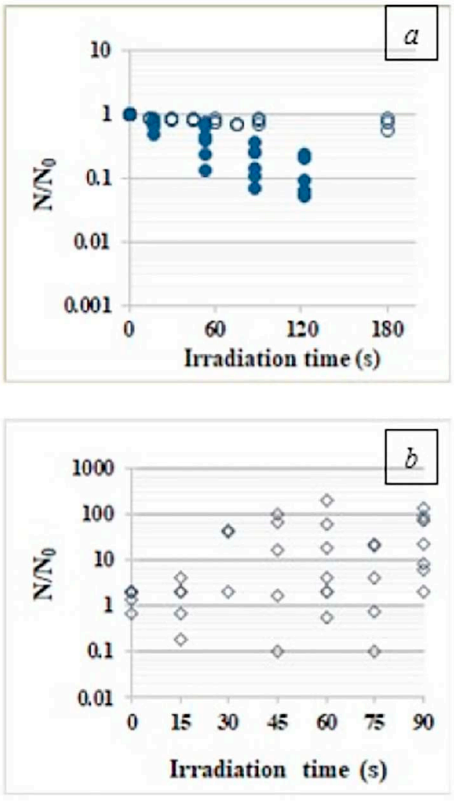


**Fig. 2.** Effect of UV-C radiation emitted in continuous (11 W/cm$^2$) ○ and in pulses (67.5 W/cm$^2$) (●) on *E. faecalis* reduction, and emitted in continuous (11 W/cm$^2$) ◊ on *D. radiodurans* reduction, both previously inoculated, one at a time, on zucchini surface.

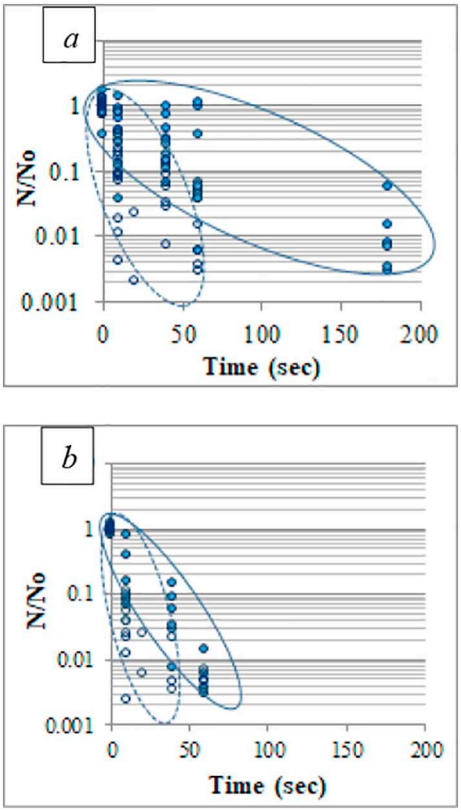


**Fig. 3.** Effect of the sequence Blanching + UV-C Pulses (●) or UV-C Pulses + Blanching (○) on *E. faecalis* reduction on zucchini surface at 60 °C (*a*) and 90 °C (*b*).

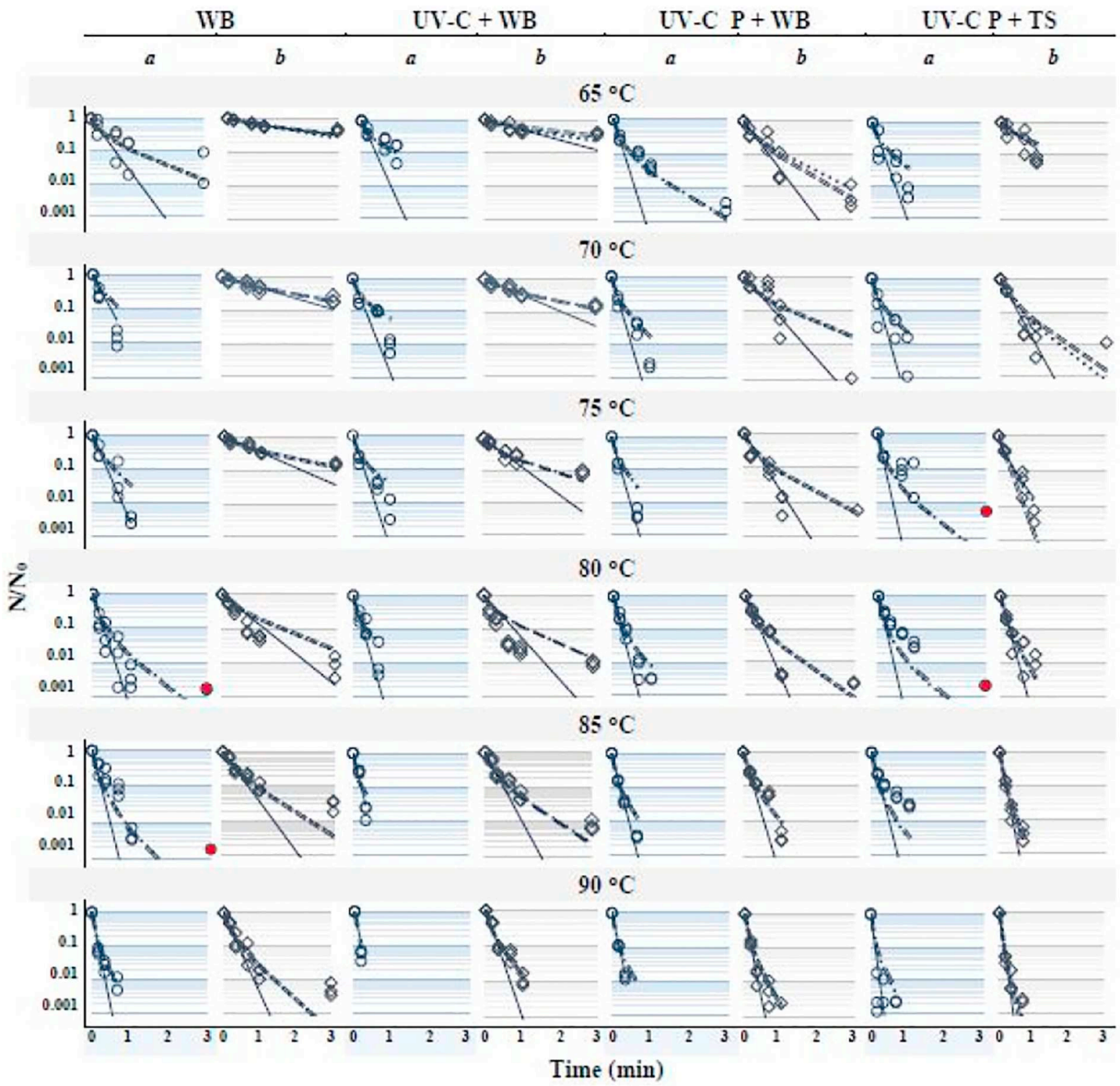


**Fig. 4.** Kinetic effect of all the pre-treatments on microbial reduction of *E. faecalis* and *D. radiodurans* previously inoculated one at a time, on zucchini through the application of blanching (WB) alone, combined with ultraviolet radiation emitted in continuous (UV-C + B), by pulses (UV-C P + WB) or thermosonication combined with UV-C pulses (UV-C P + TH). For *E. faecalis*, experimental data of survival cells (○), *D. radiodurans* experimental data of survival cells (⋄). Experimental data fitted to Bigelow model (−), Weibull-Peleg model (●●●) and modified Weibull-Mafart model (=). The best pre-treatment combinations conditions are enhanced by a red rectangle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temperatures, 85 and 90 °C, benefits are more noticeable for both *E. faecalis* (3 $\log_{10}$ reductions in < 30 s) and *D. radiodurans*. For the latter microorganism only a 1 $\log_{10}$ reduction is obtained for temperatures below 80 °C after 3 min WB, 2 $\log_{10}$ reduction at 80 and 85 °C for the same water blanching time and 2 $\log_{10}$ reduction by 2 min WB when at 90 °C. The beneficial effect of the combination of UV-C P + WB on *E. faecalis* reduction can be seen in Fig. 4, for all temperatures below 80 °C (around 1 more $\log_{10}$ reduction for all temperatures). However, the beneficial effect on the reduction of *D. radiodurans* is clear when compared to WB or UV-C + WB, being possible to obtain a 3 $\log_{10}$ reduction above 85 °C by 2 min. To date, several mechanisms have been proposed to explain pulsed UV-C light lethal effect, all of them related to the UV part of the spectrum and its photochemical effect (Wang et al., 2005; Elmnasser et al., 2007). Lasagabaster and de Marañón (2014) claimed that pulsed light causes sub-lethal damage turning bacteria cells more sensitive to the post inflicted stress such as blanching. McDonald et al. (2000) obtained similar results with *Bacillus subtilis* spores in aqueous solutions and surfaces, with pulsed UV-light the inactivation was significantly higher than continuous wave UV light. Since the intense UV-C pulses decontamination effect seems to depend on the light absorption by microorganisms, certain food could also absorb the effective wavelengths and decrease the efficacy of this treatment. Nevertheless, detailing the UV-C pulses conditions using other parameters such as pulse frequency, pulse width and polarity may help in determining the efficiency of the treatment (Aguiló-Aguayo et al., 2008; Mosqueda-Melgar et al., 2008a, 2008b, 2008c).

When replacing blanching by thermosonication combined with UV-C Pulses (UV-C P + TS) *E. faecalis* log reduction did not increase and even decreased as WB temperature increases (red dots) suggesting an antagonistic effect of ultrasounds when combined with water blanching. However, notorious benefits can be seen on *D. radiodurans* for all temperatures due most probably to mechanical effect of ultrasounds induced by the formation of pressure gradients during the collapse of cavitation bubbles within or near the bacteria caused some

destruction on the protecting protein on the cell membrane leaving them less resistant (Cruz et al., 2013).

### 3.3. Mathematical modeling

#### 3.3.1. Bigelow model

In an attempt to model mathematically the data presented, the first model used was the Bigelow model. Table 1 presents the estimated kinetic parameters for this model and Fig. 4 presents also the model curves obtained. On *E. faecalis* this model fitted well the experimental data for all treatments except UV-C + TS for all temperatures besides 65 °C where after the first 10 s some counts would decrease at a much slower rate than the ones predicted by this model. As for *D. radiodurans,* the deviation from the Bigelow model was observed for all treatments applied, besides UV-C + TS, and in the range of temperatures studied which confirms its resistance to heat and UV radiation. This behavior is in agreement with the statement of Geeraerd et al., 2005, that the log-linear model is not accurate in describing the behavior of most bacteria inactivation when undergoing both thermal and non-thermal processes. The existence of more resistant bacteria in a population can be explained by an age dependence of the cells' resistance to temperature, being old cells more liable than the younger ones (White, 1953). In fact, bacteria heat tolerance may be related to the bacteria life cycle. In the stationary phase, cells are naturally more resistant to heat than in the exponential phase (Steels and Learmonth, 1994). Barbosa-Cánovas et al. (1999) reported that cell membrane properties might vary in the different growth stages leading to different heat resistance of bacteria. This behavior was probably the reason for the deviation on microbial reduction predicted by the Bigelow model and observed for both bacteria (Fig. 4).

In addition, *E. faecalis*, known to have the ability to form biofilms, may start to adhere (starting step in biofilm formation) to the zucchini surface during the drying time (around 4 h), layer by layer and consequently the underneath layers will become less exposed to the UV-C radiation and to heat transfer (Gómez-López et al., 2005; Mohamed and Huang, 2007).

#### 3.3.2. Using the Weibull-Peleg model

Fig. 4 shows that most semi-logarithmic survival curves were clearly nonlinear, being most of them concave and with a tailing effect. For both microorganisms, the $p$ value was $< 1$ (Table 2). Therefore, following the interpretation of Albert and Mafart, 2005, the remaining cells though stressed are not very injured and may adapt to the new situation. Also, it can be noticed that $p$ presents a higher value for the blanching treatment alone when compared with the combined treatments and though this difference is not significant at 0.05% confidence level, it might indicate that the process might affect $p$, proving that this parameter is not only strain-dependent. These results confirm that a single $p$ value evaluated from a set of survival kinetics is sufficient to describe the survival kinetics, but the effect of external factors such as irradiation with UV at a high fluence on bacterial heat resistance might change it.

#### 3.3.3. Using the Weibull-Mafart model

By observing once more Fig. 4, it can be seen that the Weibull-Mafart model, though estimating other parameters, δ and z, (Table 3) presents almost the same trend as the Weibull-Peleg model for both microorganisms. Table 4 shows that the addition of UV-C radiation applied continuously (UV-C + WB) or pulsed but with thermosonication (UV-C P + TS) to the water blanching treatment, did not affect the δ value significantly but when applied in pulses (UV-C P + WB) the δ value decreased significantly meaning that this treatment is beneficial to decrease the temperature of the heat treatment. The additive effect of the ultrasound waves when added to heat is here antagonistic (Cruz et al., 2013) as the δ value increased. As for the $z$ -value, the opposite occurred, it only increased significantly at a 5% level for UV-C P + WB and UV-C P + TS, meaning that *E. faecalis* lost sensitivity to temperature changes with these two treatments. Regarding *D. radiodurans (b)*, it can be seen that δ decreases significantly at 5% level for UV-C P + WB and UV-C P + TS meaning that this microorganism became more heat labile with these two treatments, but the z-value remained unchanged meaning that this microorganism didn't increase its sensitivity significantly to temperature changes with any of the combined treatments. When zucchini was contaminated with *E. faecalis* and *D. radiodurans*, the combination of pulsed UV-C light and water blanching, proved to be, significantly more effective. Modeling of its reduction behavior allows prediction of the best conditions in new blanching processes by associating these two treatments with a lower temperature to achieve a 3 log reduction in microbial load in $< 2$ min, minimizing sensory and quality changes.

## 4. Conclusion

These results confirm the hypothesis that short UV-C in high fluences (pulses of incident irradiance of 67 W/m$^2$) combined with water blanching can be effective in preventing microbial deterioration in processed smooth surface vegetables by reducing the microbial population and thereby keeping the quality of products such as zucchini squash. Notably, these type of treatments, if efficient, can minimize the thermal impact of water blanching on vegetables to be frozen and obtain the same level of microbial reduction with a less severe heat treatment (lower temperature in a shorter time) applied due to the synergistic effect of the combined treatment of UV-C P at 67 W/m$^2$ + WB.

Considering that a vegetable or fruit with a smooth skin might be contaminated by heat resistant enterobacteriaceae and high radiation resistant microorganisms, the results from this study will help to design better pre-treatment conditions, minimizing safety threats and maximizing the product quality. These results also proved to overcome what industry is requiring so far (a 2 log microbial reduction in 3 min), hence minimizing quality changes of frozen zucchini.

**Table 2**
Overview of Bigelow model kinetic parameters estimated on zucchini surface (*Cucurbita pepo L.*) inoculated with *E. faecalis* and *D. radiodurans*.

| | *E. faecalis* | | | | | *D. radiodurans* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $D_{77,5\ °C}$ (s) | $z$ (K) | *RMSE* | $R^2$ | $R^2_{adj.}$ | $D_{77,5\ °C}$ (s) | $z$ (K) | *RMSE* | $R^2$ | $R^2_{adj.}$ |
| WB | 21 ± 1 | 44.2 ± 7.5 | 0.109 | 0.96 | 0.95 | 87 ± 4 | 23 ± 1 | 0.092 | 0.98 | 0.98 |
| UV-C + WB | 17 ± 1 | 70.37 ± 12.4 | 0.07 | 0.98 | 0.98 | 63 ± 3 | 25 ± 2 | 0.116 | 0.96 | 0.96 |
| UV-C P + WB | 14.0 ± 0.4 | 146 ± 33 | 0.034 | 0.99 | 0.99 | 29 ± 2 | 31 ± 4 | 0.126 | 0.94 | 0.94 |
| UV-C P + TS | 15 ± 1 | 158 ± 74 | 0.082 | 0.97 | 0.97 | 18 ± 1 | 35 ± 3 | 0.054 | 0.99 | 0.99 |

**Table 3**
Overview of Weibull-Peleg model kinetic parameters estimated on zucchini surface (*Cucurbita pepo* L.) inoculated with *E. faecalis* and *D. radiodurans*.

| | *E. faecalis* | | | | | | *D. radiodurans* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $k_o$ ($s^{-1}$) | Tc (K) | *p* | RMSE | $R^2$ | $R^2_{adj}$ | $k_o$ ($s^{-1}$) | Tc (K) | *p* | *RMSE* | $R^2$ | $R^2_{adj}$ |
| WB | 0.047 ± 0.007 | 396 ± 8 | 0.63 ± 0.09 | 0.093 | 0.96 | 0.96 | 0.080 ± 0.005 | 394 ± 2 | 0.73 ± 0.05 | 0.082 | 0.98 | 0.98 |
| UV-C + WB | 0.041 ± 0.005 | 388 ± 5 | 0.512 ± 0.046 | 0.055 | 0.99 | 0.98 | 0.072 ± 0.006 | 392 ± 3 | 0.66 ± 0.06 | 0.103 | 0.97 | 0.97 |
| UV-C P + WB | 0.032 ± 0.009 | 398 ± 14 | 0.446 ± 0.072 | 0.071 | 0.98 | 0.98 | 0.059 ± 0.008 | 390 ± 5 | 0.66 ± 0.08 | 0.116 | 0.95 | 0.95 |
| UV-C P + TS | 0.02 ± 0.003 | 420 ± 16 | 0.716 ± 0.056 | 0.074 | 0.98 | 0.98 | 0.059 ± 0.004 | 386 ± 3 | 0.70 ± 0.05 | 0.054 | 0.99 | 0.99 |

**Table 4**
Overview of a modified version of Weibull-Mafart model kinetic parameters estimated on zucchini surface (*Cucurbita pepo* L.) inoculated with *E. faecalis* and *D. radiodurans*.

| | *E. faecalis* | | | | | | *D. radiodurans* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_{77.5\ °C}$ (s) | *z* (K) | *p* | *RMSE* | $R^2$ | $R^2_{adj.}$ | $\delta_{77.5\ °C}$ (s) | *z* (K) | *p* | RMSE | $R^2$ | $R^2_{adj.}$ |
| WB | 28 ± 4 | 33 ± 6 | 0.67 ± 0.08 | 0.104 | 0.96 | 0.96 | 123 ± 11 | 21 ± 1 | 0.73 ± 0.05 | 0.082 | 0.98 | 0.98 |
| UV-C + WB | 25 ± 3 | 32 ± 5 | 0.49 ± 0.06 | 0.069 | 0.99 | 0.98 | 95 ± 11 | 22 ± 2 | 0.66 ± 0.06 | 0.102 | 0.97 | 0.97 |
| UV-C P + WB | 17 ± 1 | 64 ± 10 | 0.55 ± 0.05 | 0.034 | 0.99 | 0.99 | 38 ± 4 | 27 ± 3 | 0.66 ± 0.08 | 0.108 | 0.95 | 0.95 |
| UV-C P TS | 23 ± 3 | 73 ± 7 | 0.45 ± 0.07 | 0.073 | 0.98 | 0.98c | 21 ± 1 | 29 ± 2 | 0.70 ± 0.05 | 0.048 | 0.99 | 0.99 |

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