Área 3: Alimentos do século XXI: matérias-primas, processos e produtos

# Processing zucchini (*Cucurbita pepo L.)* with low UV-C radiation

PORTELA, Mauro[1]; SILVA, Cristina L.M.[2];BRANDÃO, Teresa R.S.[2]; GONÇALVES, Elsa M.[3]; ABREU, M.[3]; OLIVEIRA, Antonio J.[4], Gaspar, Nelma P.[1] VIEIRA, Margarida C.[1]

[1] Escola Superior de Tecnologia – Universidade do Algarve, Campus da Penha, 8005-139 Faro.
[2] Escola Superior de Biotecnologia – Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto.
[3] Departamento das Indústrias Alimentares – Instituto Nacional de Engenharia, Tecnologia e Inovação, Estrada do Paço do Lumiar, 1600-038 Lisboa.
[4] Friopesca – Refrigeração de Aveiro, S.A. Av. Pedro Álvares Cabral – Apart.9, 3834-908 Gafanha da Nazaré.

E-mail: mvieira@ualg.pt

## Abstract

Recent developments regarding the antimicrobial properties of certain types of light sources have promoted a growing interest in these applications, especially with concern to their potentialities for food processing. Ultraviolet radiation in the range of 200-280 nm is lethal to most microorganisms, including bacteria, viruses, fungi and yeasts. Fresh and artificially contaminated zucchini (*Cucurbita pepo L.*), whole and cut into triangular prisms, was exposed to UV-C lamps (Philips TUV-TL mini 8W) for different periods of time with the objective of quantifying the germicidal effect of this treatment. Triangular prism cut samples of zucchini were processed for 5, 7.5, 10, 12.5 and 15 min with different energy discharges of UV-C radiation, respectively 2.1 and 8.4 watts, whereas whole zucchini were strategically exposed for maximum time/radiation conditions (15 min - 8.4W) due to larger surface area treatments.

In freshly cut samples, total counts of mesophilic bacteria were assayed, although in the case of whole vegetables, these were initially inoculated with commercial strains of *Enterococcus faecalis* ATCC 29212, and consequently assayed for presence of this specific microorganism.

In both cases, UV-C exposure significantly reduced microbial activity, however, in relation to the freshly cut samples, after initial reduction of mesophilic bacteria, observations of photoreactivation between 7.5 and 12.5 min were registered, with significant counts at 10 min. Decrease in bacterial growth was once again evidenced between 10 and 12.5 min but not confirmed at 15 min of exposure. As for whole contaminated vegetables, approximately 2 logarithmic reductions of the target microorganism (*Enterococcus faecalis* ATCC 29212) were achieved.

**Keywords:** zucchini, germicidal effect; UV-C radiation; photoreactivation

## 1. Introduction

Food Safety has become an important issue among food companies these days and a definite requisite for consumers especially in an efficiently controlled food safety farm-to-fork concept. Documented cases of various foodborne illness associated with fresh produce have increased substantially in recent years and major outbreaks have been linked to contamination by common foodborne pathogens such as *Salmonella*, *Listeria monocytogenes*, *Shigella spp.*, *Escherichia coli* and *Enterococcus faecalis*.
Although current techniques in processing fresh vegetables have improved the overall quality and extended shelf life there is a need for novel preservation practices for fresh processed vegetables quality attributes during all the distribution chain (Allende et al, 2003). Recent developments on antimicrobial properties of certain types of light sources promoted a growing interest in these applications in food processing.
Ultraviolet radiation, UV-C, (200-280 nm) is sometimes called the germicidal range due to being lethal to most microorganisms. Numerous studies have demonstrated the effectiveness of low UV-C radiation from germicidal lamps in reducing deterioration of produce by reducing microbial spoilage and putrefaction on onions (Lu *et al*, 1987), carrots (Mercier and Arul, 1993), tomatoes (Liu *et al*, 1993), red apples (Yaun, *et al*, 2004), strawberries (Marquenie et al., 2002), table grapes (Nigro *et al*, 1998) and lettuce (Allende *et al*, 2003).
UV-C radiation is used in many ways in a food processing plant, namely for disinfection of contact surfaces, rinsing water or air in a preparation area. The equipment is relatively inexpensive, but operation is subject to certain safety precautions. (Bintsis *et al*, 2000). High intensity UV-C lamps have become widely available and their destruction potential for surface bacteria on foods has been enhanced by concentrated peak radiation at 253.7 nm, a significant germicidal point of the electromagnetic spectrum.
UV-C involves direct alteration of microbial DNA due to its UV light absorption, causing cross-linking between neighbouring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand. Due to the mutated base, formation of the hydrogen bonds to the purine bases on the opposite strand is impaired. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death. The amount of cross-linking is proportional to the amount of UV exposure. The level of mutations that can be reversed depends on the ability of target microorganisms to repair the photochemical damage to DNA (photoreactivation). Once exceeded the threshold of cross-linking, cell death occurs (Miller *et al*, 1999).
The general shape of the curve for microbial inactivation by UV light is sigmoidal, having an initial plateau due to an injury phase of the microorganism in response to UV exposure. After that, minimal additional UV exposure would be lethal for microorganisms and survivor numbers rapidly decline being the end a tailing phase due to the resistant microorganisms.
The overall objective of this study was to analyze the bactericidal effects of UV-C light at 253.7 nm on zucchini and to evaluate its future potential as a microbiological hurdle on fresh and processed cucurbitaceous vegetables.

## 2. Material and methods

### *2.1 Sample preparation*

Fresh zucchini *(Cucurbita pepo L.)* where purchased daily from local suppliers. To study surface contamination of the product, both conical tips of the vegetable were discarded (fig.1) and samples were cut in triangular prisms of approximately 10 grams, from the surface inwards (fig.2).

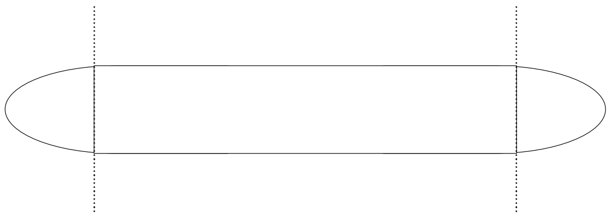

**Fig. 1 –** Conical sides of the vegetable are cut and discarded

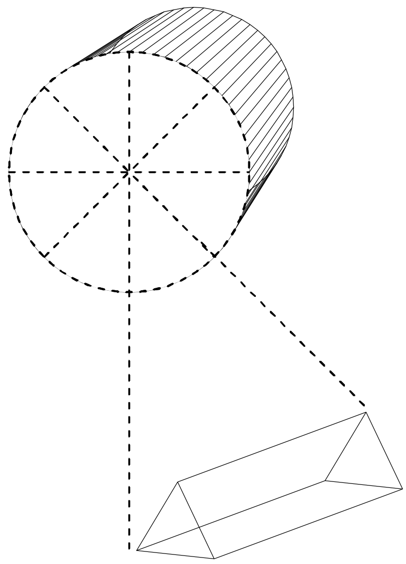

**Fig. 2 –** Triangular prism samples are cut from surface inwards

*2.2 UV-C treatment*

The UV-C disinfection chamber consisted of rectangular box, built with an extremely resistant and lightweight alloy, Dibond® which is a three layer laminate Aluminum/LDPE polyethylene/Aluminum. Four TUV TL mini 8W lamps (Philips) were suspended horizontally inside the chamber. Samples were placed on an acrylic tray at 30 cm from the UV-C lamps to simulate a processing line. To avoid temperature increases, a fan was placed on the top section of the chamber, above the lamp bank. Although the lamps have 8 watts of nominal power, only about 1/4 of this power is considered effective UV-C radiation. Ultraviolet radiation doses varied, by turning off individual lamps and by altering the duration of the exposure time.

Triangular prism cut samples of zucchini were immediately processed for 5, 7.5, 10, 12.5 and 15 min with different energy discharges of UV-C radiation, respectively 2.1 and 8.4 watts, whereas whole zucchini were strategically exposed for maximum time/radiation conditions (15 min - 8.4W) due to larger surface area treatments.

The different UV-C treatments performed in the present study are further described in Fig. 3, along with the respective microbiological enumerations performed for each type of sample.

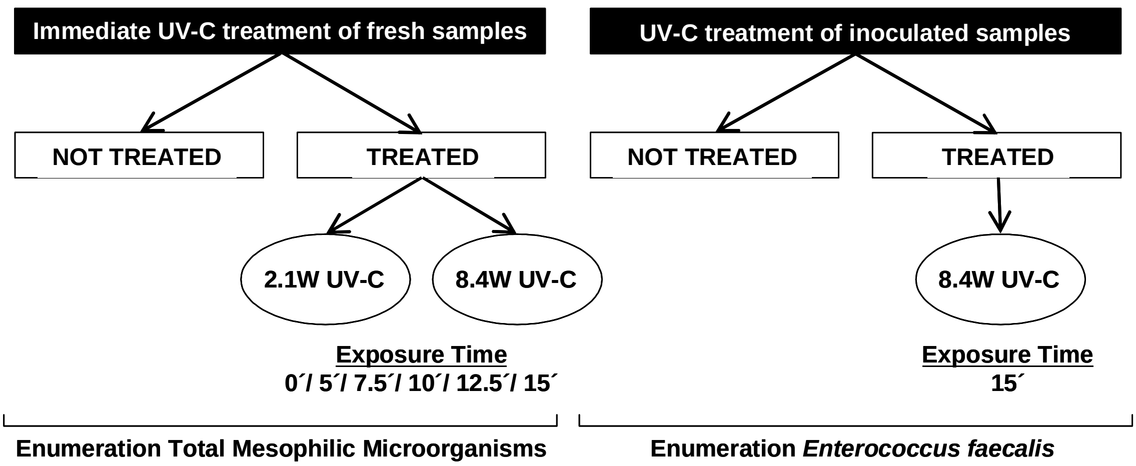


**Fig. 3 –** Overall combinations of radiation doses and exposure times studied

*2.3 Inoculum cocktail of E. faecalis*

Strains of *Enterococcus faecalis* ATCC 29212 were acquired commercially in the Culti-loop® form. Strains was firstly inoculated onto sterile petri dishes containing approximately 15 mL of plate count agar (PCA 01-161, Scharlau) and incubated for several hours at 37ºC in order to obtain a significant number of viable cells. Once viability had been observed and tested, strains were further inoculated into 250 mL of brain heart infusion broth (BHI, Oxoid) growth medium and incubated again at 37ºC for exactly 15 hours. Turbidity tests were run using a common laboratory spectrophotometer in order to achieve an optical density reading between 0.21-0.24 at 620nm. In cases of excessive growth and high turbidity levels, dilutions were performed in order to obtain readings in the referred target range. An inoculum cocktail was then prepared by adding 10 mL of *E. faecalis* microbial cell suspension in BHI to precisely 1 L of sterile distilled water.
Whole zucchini were slowly dipped into the previously prepared inoculum cocktail and maintained beneath liquid level for approximately 10 min.

*2.5 Enumeration of E. faecalis*

After *E. faecalis* growth was quantified by turbidity tests, parallel enumeration of colonies per millilitre of growth medium were attained by spread plating with slanetz and bartley medium (SBM, Oxoid). UV-C treated produce was aseptically sampled by cutting triangular prism samples of approximately 10 grams from different parts of the vegetable. Samples were transferred to sterile stomacher bags and subject to homogenization in 90 mL of buffered peptone water (BPW 02-277, Scharlau), for 6 min. Serial dilutions of 1:10 in BPW were performed and spread plating was managed with slanetz and bartley medium (SBM, Oxoid). Plates were incubated at 35ºC for 4 hours and then at 44-45ºC for 44 hours.

*2.6 Enumeration of total mesophilic microorganisms*

Enumeration of total mesophilic aerobic microorganisms on zucchini pieces was performed by using a standard method (NP-1995). Samples of 10 g from each treatment were homogenised in a sterile stomacher bag in 90 mL of sterile buffered peptone water (BPW 02-277, Scharlau) for approximately 6 min. Ten-fold dilution series were made in BPW, as needed for plating. The following media and incubation conditions were used: incorporation into plate count agar (PCA 01-161, Scharlau); inverted plate incubation for 72 hours at 30ºC.

## 3. Results and discussion

*3.1 Mesophilic microorganisms*

Total populations of mesophilic microorganisms present in *Cucurbita pepo* samples were generally reduced by UV-C exposure especially at stronger doses of UV-C radiation and maximum exposure times. However, effects of ultraviolet exposure did not have a linear evolution with respect to exposure time, especially between 7.5 and 12.5 min (Fig. 4). Although fresh produce had significantly different initial contamination levels of mesophilic bacteria throughout the experimental analysis, it is safe to say that doses of 8.4 W UV-C radiation had clearly more damaging effects at an initial phase, than at 2.1 W UV-C. Microbial populations present showed significant resistance to lower doses of UV-C radiation, and only above 10 min of exposure time did enumerated colonies start to diminish. Nevertheless, after 12.5 min results were not conclusive between tests.

Stronger UV-C doses of approximately 8.4 W showed effective lethality on microbial counts especially until 5 min exposure. At 7.5 min, microbial load began to increase reaching a peak point at 10 min of exposure, after which growth seemed to decrease once again until 12.5 min. Between 12.5 and 15 min, the same behaviour was also observed, although on a much smaller scale, giving a sort of tail end to the microbial inactivation curve. This behaviour may support the prediction that at certain levels of UV-C exposure, surviving or injured bacteria may have the ability to resist ultraviolet light and repair damaged DNA by photoreactivation. This type of behaviour by indicator and non-human pathogenic microorganisms following UV disinfection has been subject to many studies although no truly conclusive models were developed to explain this phenomenon (Tosa *et al*, 1998). It was however hypothesized that this type of behaviour may vary somewhat between different microorganisms.
Results obtained where somewhat different to those expected as the inactivation curve for total mesophilic microorganisms was not a simple sigmoidal curve but moreso a combination of sigmoidal curves throughout the process.

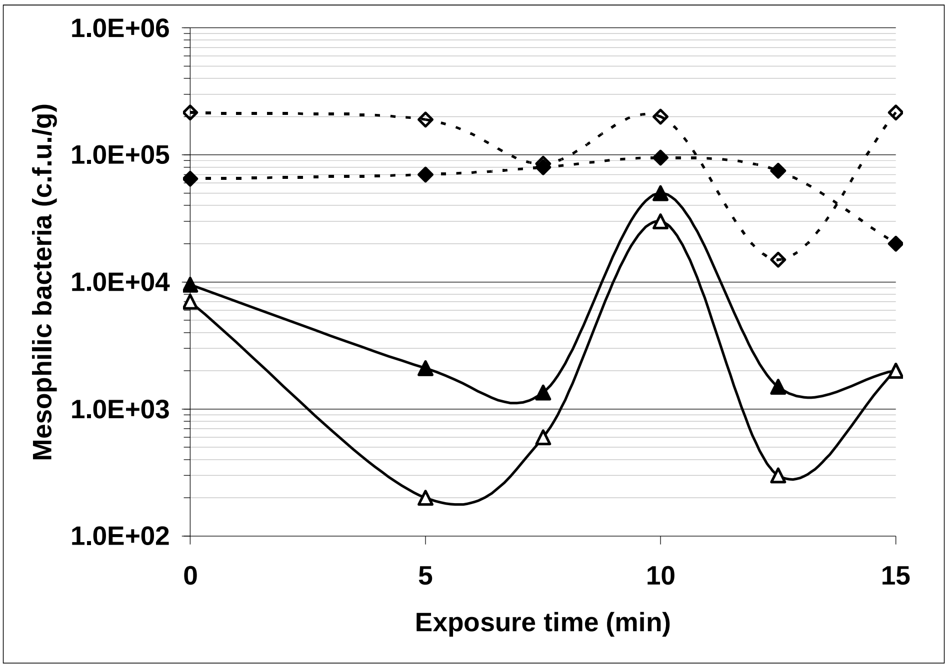


**Fig. 4** – Mean counts of total mesophilic aerobic on freshly cut zucchini samples at different UV-C treatments: continuous lines – 2.1 W UV-C; dashed lines -- 8.4 W UV-C

### *3.2 Enterococcus faecalis ATCC 29212*

Artificially contaminated samples of whole *Cucurbita pepo* vegetables were subject to 8.4 W of UV-C radiation during exactly 15 min. Objective evidence of UV-C lethality with regard to *E. faecalis* was undoubtedly confirmed as the data collected from microbiological enumeration showed that approximately 2 logarithmic reductions of this target microorganism were achieved (Fig. 5).
Despite no outputs regarding photoreactivation ability during the 15 minute period of exposure, these results are positive with regard to two aspects: 1) as far as we know, no previous studies of UV-C inactivation had clearly focused on *E. faecalis*; 2) *E. faecalis*, normally an indicator of faecal contamination and considered in past years as the main target microorganism during the design of UV disinfection processes for natural mineral water (Urakami *et al*, 1997) may be of importance with regard to the design of UV disinfection processes for fresh produce.

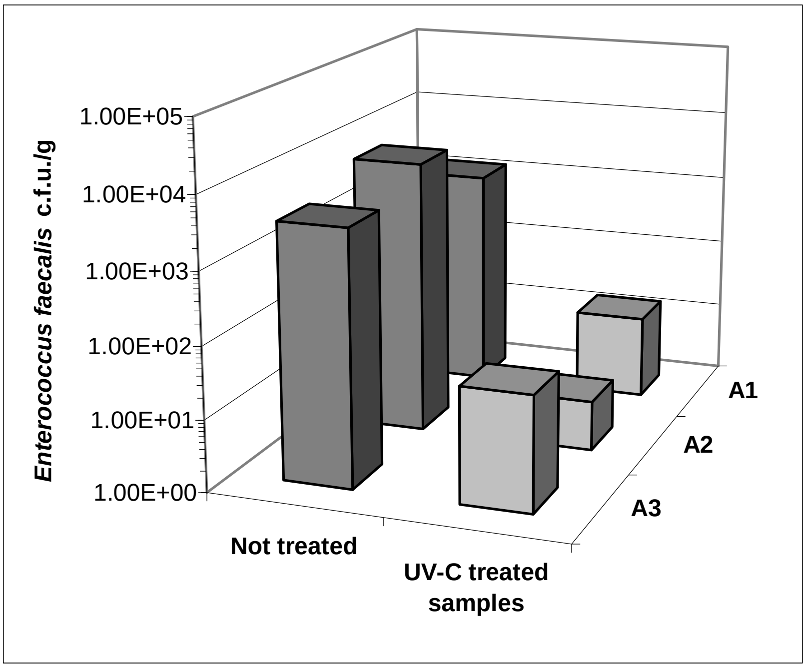


**Fig. 5 –** Mean microbial counts of *E. faecalis* achieved with whole zucchini after artificial contamination: no treatment – grey bares; treated with 8.4 W UV-C for 15 min – light-grey bares

## 4. Conclusions

In most samples analyzed, UV-C exposure significantly reduced microbial activity, however, considering the freshly cut samples, after initial reduction of mesophilic bacteria, a photoreactivation between 7.5 and 12.5 min was registered, with significant counts at 10 min. Decrease in bacterial growth was once again evidenced between 10 and 12.5 min but not confirmed at 15 min of exposure.

It becomes evident that the number of microorganisms with photoreactive ability may decrease substantially with prolonged exposure times (as observed by short tailing at the end of inactivation graphs) although further studies are welcome on this issue.

For whole contaminated vegetables, approximately 2 logarithmic reductions of the target microorganism (*Enterococcus faecalis* ATCC 29212) were achieved.

Our present findings reveal that due to dispersed results obtained when assaying total populations of mesophilic microorganisms, and considering that effects of UV-C light may vary somewhat between different microorganisms, the present work may serve as evidence that indicator micrорganisms and pathogens alike should be individually studied in relation to UV-C inactivation. Microbial reduction can also be correlated with precise UV-C radiation doses ($mW/cm^2$) in order to confirm results attained in the present work.

Another issue was enhanced in this study, photoreactivation should definitely be taken into consideration to a large extent during the design of the UV-C disinfection process, especially at low radiation doses and short exposure times.

The authors acknowledge the financial support through Programa Operacional Agricultura e Desenvolvimento Rural – Projecto AGRO nº822 (Novas Tecnologias de Processamento de Hortofrutículas Congelados – EMERCON).

## 5. References


ALLENDE, A.; ARTÉS, F. 2003. UV-C radiation as a novel technique for keeping quality of fresh processed 'Lollo Rosso' lettuce. *Food Research International*, 36, 739–746.

BINTSIS, T.; LITOPOULOU-TZANETAKI, E.; ROBINSON, R. K. 2000. Existing and potential applications of ultraviolet light in the food industry - a critical review. *Journal of the Science of Food and Agriculture*, 80, 637–645.

LIU, J.; STEVENS, C.; KHAN, V. A.; LU, J. Y.; WILSON, C. L.; ADEYEYE, O.; KABWE, M. K.; PUSEY, P. L.; CHALUTZ, E.; SULTANA, T.; DROBY, S. 1993. Application of ultraviolet-C light on storage rots and ripening of tomatoes. *Journal of Food Protection*, 56(10), 868–872.

LU, J. Y.; STEVENS, C.; YAKABU, P.; LORETAN, P. A.; EAKIN, D. 1987. Gamma, electron beam and ultraviolet radiation on control of storage rots and quality of Walla Walla onions. *Journal of Food Processing and Preservation*, 12, 53–62.

MARQUENIE, D.; MICHIELS, C. W.; GEERAERD, A. H.; SCHENK, A.; SOONTJENS, C.; VAN IMPE, J. F.; NICOLAÏ, B. M. 2002. Using survival analysis to investigate the effect of UV-C and heat treatment on storage rot of strawberry and sweet cherry. *International Journal of Food Microbiology*, 73, 187–196.

MERCIER, J.; ARUL, J.; JULIEN, C. 1994. Effect of food preparation on the isocoumarin, 6-methoxymellein, content of UV-treated carrots. *Food Research International*, 27: 401–404.

MILLER, R.; JEFFREY, W.; MITCHELL, D.; ELASRI, M. 1999. Bacterial responses to ultraviolet light. *American Society of Microbiology*, 65(8), 535–541.

NIGRO, F.; IPPOLITO, A.; LIMA, G. 1998. Use of UV-C light to reduce Botrytis storage rot of table grapes. *Postharvest Biology and Technology*, 13, 171–181.

TOSA K.; HIRATA T. 1999. Photoreactivation of enterohemorrhagic Escerichia coli following UV disinfection *Water Research* - Vol. 33, No. 2, pp. 361-366.

URAKAMI I.; YOSHIKAWA M.; UDAGAWA J.; SUGAHARA T. 1997. Ultraviolet light disinfection of natural mineral water. *Journal Antibact Antifung Agents – Japan*, 25, 697-701.

YAUN, B. R.; SUMNER, S. S.; EIFERT, J. D.; MARCY, J. E. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. *International Journal of Food Microbiology*, 90, 1–8.