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Ultraviolet light and Ultrasound as non-thermal treatments for the inactivation of microorganisms in fresh ready-to-eat foods

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ABSTRACT

The effects of two non thermal disinfection processes, Ultraviolet light (UV 254 nm) and Ultrasound (US) on the inactivation of bacteria and color in two freshly cut produces (lettuce and strawberry) were investigated. The main scope of this work was to study the efficacy of UV and US on the decontamination of inoculated lettuce and strawberries with a cocktail of four bacteria, *Escherichia coli*, *Listeria innocua*, *Salmonella* Enteritidis and *Staphylococcus aureus*. Treatment of lettuce with UV reduced significantly the population of *E. coli*, *L. innocua*, *S.* Enteritidis and *S. aureus* by 1.75, 1.27, 1.39 and 1.21 log CFU/g, respectively. Furthermore, more than a 2-log CFU/g reduction of *E. coli* and *S.* Enteritidis was achieved with US. In strawberries, UV treatment reduced bacteria only by 1–1.4 log CFU/g. The maximum reductions of microorganisms, observed in strawberries after treatment with US, were 3.04, 2.41, 5.52 and 6.12 log CFU/g for *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua*, respectively. Treatment with UV and US, for time periods (up to 45 min) did not significantly ($p > 0.05$) change the color of lettuce or strawberry. Treatment with UV and US reduced the numbers of selected inoculated bacteria on lettuce and strawberries, which could be good alternatives to other traditional and commonly used technologies such as chlorine and hydrogen peroxide solutions for fresh produce industry. These results suggest that UV and US might be promising, non-thermal and environmental friendly disinfection technologies for freshly cut produce.

1. Introduction

Sales of fresh produce have significantly been increased during the last decade, as consumers become increasingly concerned with healthy food and nutrition (Organic Trade Association, 2008). Fruit and vegetables are considered important components of a healthy diet, because of their content in vitamin C, phenolic compounds, fiber content, anthocyanins, flavonols (Mulabagal et al., 2010; Odriozola-Serrano et al., 2010). Their daily consumption could help preventing major diseases, such as cardiovascular diseases and certain types of cancers (Piyasena et al., 2003). Considering the global market, Greece as a Mediterranean country is among the most important fruit and vegetable producers of the world (Moreno and Fereres, 2012).

Fresh and freshly cut produces are responsible for a growing number of foodborne disease outbreaks each year (Beuchat, 2002). The number of outbreaks, caused by foodborne pathogens, has also increased because of the increased consumption of minimally processed fresh produce (Sivapalasingam et al., 2004). Fresh produces can be contaminated with foodborne pathogens while growing in fields or orchards, or during harvesting, postharvest handling, processing, and distribution (Beuchat, 1996). Sources of microbial contamination on fruits and vegetables during production include animal and human feces, contaminated manure, inorganic amendments, irrigation water, water used for pesticide application or other agricultural purposes and contaminated dust (Beuchat and Ryu, 1997). The minimal processing of these products makes it difficult to ensure that fresh produce is safe for consumers. Moreover, tissue damage during processing and subsequent release of nutrients in freshly cut produce enhances microbial development (Harris et al., 2003). Numerous outbreaks in a wide variety of ready-to-eat foods have also been reported due to extensive human handling during their preparation or by cross-contamination from the environment (Escalona et al., 2010). *Listeria* spp., *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* are the most common pathogens detected in vegetables and fruits (Beuchat, 1996; Bracket, 1999). The lack of any heating step prior to consumption of the fresh produce, emphasizes the need for taking sanitary measures for the prevention and reduction of the microbial load, but simultaneously maintaining the "fresh" status and the organoleptic properties of the produce, intended to be consumed raw (Baert et al., 2009). Disinfection remains one of the most important critical points along the processing line, in order to ensure the safety and quality of freshly cut vegetables and fruits for a defined period of shelf-life (Beuchat et al., 1998; Bachmann and Earles, 2000). However, a detrimental effect on nutritional properties and perceived quality of the fresh produce has

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been reported. Therefore, researchers are investigating new non thermal methods to reduce pathogens and simultaneously to ensure the safety and quality of the produce. Non-thermal technologies, such as ozonation, ultrasonication (US) and ultraviolet light (UV) have been applied to an extensive variety of food products to destroy microorganisms associated with spoilage and contamination (Cao et al., 2010; Hernández et al., 2010; Ölmez and Akbas, 2009).

The inactivation mechanism of UV is the formation of photoproducts in the DNA. Of these photoproducts, the most important is the pyrimidine dimer, which is formed between adjacent pyrimidine molecules on the same strand of DNA and can interrupt both DNA transcription and translation (Franz et al., 2009). Microorganisms can find protective sites in some product surfaces (e.g. lettuce, carrots) and can migrate to these sites when UV radiation is applied. The DNA damage inflicted by UV-C radiation leads to lethality by directly altering microbial DNA through dimer formation between neighboring pyrimidine nucleoside bases in the same DNA strand (Bintsis et al., 2000; Yaun et al., 2004).

Power US is defined as pressure waves, with a frequency of 20 kHz or more, which cause chemical and physical changes in biological structures (in a liquid medium) due to intracellular cavitation (Butz and Tauscher, 2002). Microbial inactivation by US is mainly due to breakage of the cell walls, disruption and thinning of cell membranes, and DNA damage via free radical production (Hulsmans et al., 2010; Scouten and Beuchat, 2002).

Both technologies are perceived to be safe, non-toxic and environmental friendly (Feng et al., 2011). Moreover, they have high efficiency, low instrumental requirements, significantly reduced process time and their performance is economically viable (Suslick, 1990).

Lettuce's color is due to the fact that this product is a complex system of enzymes, pigments and other compounds, such as peroxidase, ascorbic acid, carotenoids and chlorophyll that affect its color (Bermúdez-Aguirre and Barbosa-Cánovas, 2013). Fruit color is a major determinant of quality in red berry fruits such as strawberry and is due to the presence of anthocyanins, a group of water-soluble pigments with antioxidant properties (Patras et al., 2009). The main anthocyanin present in strawberry is pelargonidin-3-glucoside (Zabetakis et al., 2000) and recent work has suggested involvement of anthocyanins in various health benefits and cancer prevention (Zhang et al., 2004). Maintaining the natural color in freshly-cut foods is of paramount importance during food processing (Rodrigo et al., 2007).

The objective of this study was to assess two non-thermal technologies (UV and US) during different time intervals for the disinfection of *E. coli*, *Salmonella* Enteritidis, *Listeria innocua* and *S. aureus* in two different fresh produces (leafy vegetable and strawberry). In parallel, objective was to evaluate the effect of these methods (UV and US) to the food quality and color.

2. Material and methods

2.1. Food samples

Leafy green vegetables such as romaine lettuce (*Lactuca sativa L. var. longifolia*) and strawberries (*Fragaria* x *ananassa*) were selected to study the level of decontamination. They were selected as they are vegetables and fresh fruits, commonly consumed in Mediterranean diet as they ensure an adequate intake of vitamins, fibers and antioxidants. Fresh produces were purchased from a local supermarket (Patras, Greece) the day of the experiment and stored under refrigerated conditions (4 °C) for 1 h until the time of the experiment.

2.2. Bacterial strains

Bacterial strains used were *E. coli* NCTC 9001, *S. aureus* NCTC 6571, *S.* Enteritidis NCTC 6676 and *L. innocua* NCTC 11288 (HPA, Colingdale, U.K.). Lenticules with the microorganisms were rehydrated in 9 mL of peptone saline (0.1%) (Oxoid, U.K.) and after 20 min, working cultures were streaked onto Tryptic Soy Agar (TSA; Oxoid, U.K.), incubated at 37 °C for 24 h, and stored at 4 °C.

2.3. Culture preparation

Each bacterial type was cultured in 20 mL Tryptone Soya Broth (TSB; Merck, U.K.) at 37 °C for 17 h, harvested by centrifugation at 4000 ×*g* for 20 min at 4 °C and washed three times with buffered peptone water (BPW; Oxoid). The final pellets were resuspended in BPW, corresponding to approximately 10^8–10^9 CFU/mL. Bacterial cocktails were prepared by mixing equal volumes of each of the test bacterial type and tested before use.

2.4. Sample inoculation

All vegetables were rinsed with sterile water to remove some of the natural flora or any other matter before treatment. For lettuce, two to three outer leaves were discarded and the intact internal leaves were removed and weighted to give samples of 10 g. Likewise pieces of whole strawberries without calyx were weighted to give final weight of 10 g. A spot-inoculation method was used to inoculate the pathogenic bacteria on lettuce leaves and strawberry pieces (Mahmoud, 2010).

Briefly, 100 μL (10 drops) of bacterial cocktail corresponding to 10^7–10^8 of each bacteria type was spotted with a micropipette on the surface of each produce. The bacteria cocktail was evenly applied throughout the skin surface of the strawberry, approximately midway between the calyx and cap (Bialka et al., 2008). In lettuce, the cocktail was placed to the center (abaxial) outer surface of the lettuce, in order to simulate real conditions that can occur when contaminated compost and irrigation water can be transferred to lettuce leaves (Oliveira et al., 2011). 100 μL (10 drops) of deionized sterile water was spotted on the surface of control samples. To allow bacterial attachment, the samples were air dried on sterile aluminum foil in a class II biosafety cabinet (Cytair 155, FluFrance) for 2 h in 25 °C prior to treatments. The time for the surface drying was in agreement with other studies (Sagong et al., 2011). The fact that the inoculums were attached to the vegetable surfaces was verified by comparing the results with a control sample containing food only in BPW for 60 min.

2.5. Disinfection treatments

2.5.1. Ultraviolet light

For UV treatment, a UV cabinet with four UV-C (Osram Germicidal G5) lamps was used. The peak emission of the lamps was 254 nm. The inoculated samples were placed in sterile petri dishes and were left in 8 cm distance from the lamps and treated for 10, 20, 30, 45 and 60 min. As strawberries are concerned, 1–2 strawberries were cut in such a way as only their equatorial zone was exposed to UV source and their flesh was hidden. This was chosen as the purpose of the disinfection method, was to disinfect the skin of the strawberry (Aday et al., 2013). The treatment was conducted at an intensity of 2 mW/cm^2 at dosages 1.2, 2.4, 3.6, 5.4 and 7.2 J/cm^2. Throughout the experiments, the UV-C light intensity was kept constant, and the applied doses varied by altering the exposure time at the fixed distance (López-Rubira et al., 2005). Sample temperature was monitored using a K-type thermocouple attached to a Grant Data Logger (Squirrel 2040; Grant Instruments) to ensure that at the end of the longest exposure time, temperature was at 25 ± 2 °C. At least three replicates of each treatment were carried out.

2.5.2. Ultrasound

For the US treatment, a 5.75 L ultrasound tank (Elmasonic, Germany) was filled with 3 L of distilled water and used at an operating frequency of 37 kHz and a power up to 30 W/L. A glass beaker (600 mL) was placed in the US tank and filled with 9-fold dilution of BPW. The ratio fruit (strawberry) or vegetable (lettuce) to BPW for the ultrasound treatments was 1 part of food (10 g) to 9-fold dilution of BPW. Inoculated lettuce

leaves and strawberries were immersed in the glass beaker and treated with US for 10, 20, 30, 45 and 60 min. At least three replicates of each treatment were performed.

2.6. Bacterial enumeration

For enumeration of bacteria, 10 g of treated lettuce or strawberry sample was transferred into a sterile stomacher bag (Gosselin SM2B-01, Villeurbanne, France) containing 90 mL of Peptone Buffer water (PBW; Oxoid, U.K.) and homogenized in a stomacher (BagMixer, Interscience, St Nom la Bretêche, France) for 2 min. The decision for which part to analyze, was taken at the inoculation stage according to suggestions of previous researchers. 10 g of lettuce or strawberry was inoculated (according to ISO 6887-1:1999, the minimum volume of a representative test sample is 10 g), treated with disinfection technologies and finally analyzed (Bialka et al., 2008; Oliveira et al., 2011; Sagong et al., 2011). One milliliter of the homogenized sample was then 10-fold serially diluted in 9 mL of sterile BPW (0.1%), and appropriate dilutions were pour-plated into appropriate selective media. All samples were analyzed according to ISO standard methods. More specifically, Chromocult TBX plates were incubated at 44 ± 1 °C according to ISO, 16649-2:2001 for *E. coli* analysis. Baird Parker agar base plates were incubated at 37 ± 1 °C according ISO 6888-1:1999 for Staphylococci analysis. XLD plates were incubated at 37 ± 1 °C according to ISO 6579:2002 for Salmonella analysis. Listeria agar base plates were incubated at 37 ± 1 °C according to ISO, 11290:1996 for Listeria analysis. Reductions of bacteria were calculated on a per gram of fruit and vegetable basis.

2.7. Color measurement

A colorimeter (Hunterlab D25, Hunter Associates Laboratory, Inc., Reston, Virginia) was used for color measurements. The UV and US treated samples were surface dried; they were positioned then in a plastic bag and held on ice until all experiments have been completed. A lettuce and a strawberry piece (10 g) were then placed directly on the colorimeter sensor and measured in at least three different points of the product. Three measurements were performed per treatment and results were averaged. The recorded tristimulus values X, Y, and Z were converted to CIE L*, a* and b* color values. The L* parameter shows lightness to darkness and ranges from 0 (black) to 100 (white). The a* parameter measures the degree of redness (+a*) or greenness (−a*). The b* parameter measures the degree of yellowness (+b*) or blueness (−b*). The net color difference (ΔE*) and chroma or saturation index (C*) were determined using L*, a* and b* values, comparing them with the values of unprocessed samples (Bermúdez-Aguirre and Barbosa-Cánovas, 2013).

2.8. Statistical analysis

All experiments were carried out in triplicates. During each experiment four samples were taken from each vegetable at any time to conduct microbial counts and color readings. The microbiological data were analyzed in terms of log (N/N_0), where N is the microorganism load at a given time, and N_0 corresponds to the initial microbial load of untreated samples. Data on quality attributes (color) was normalized in relation to values obtained for untreated samples.

The data for inactivation of *E. coli*, *S. aureus*, *S.* Enteritidis, *L. innocua* and by UV-C and US were analyzed for statistical significance using SPSS 20.0 (SPSS Inc., Chicago, USA). Results were compared by an analysis of variance followed by Tukey's method with a significance level of $p < 0.05$.

3. Results and discussion

This study was performed in order to compare the effectiveness of two non thermal disinfection techniques, US and UV, on their ability to reduce the numbers of four different bacterial types, *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua*, inoculated on two different fresh-produces (lettuce and strawberries). *E. coli* and *L. innocua* were selected as indicators and surrogates of pathogens *E. coli* O157:H7 and *Listeria monocytogenes* respectively. *S. aureus* and *S.* Enteritidis were selected as they are common pathogens that can be found in ready-to-eat products.

Spot inoculation was the method used to inoculate the bacteria on foods, as it is more consistent and produces more reproducible results for the inoculation of a known number of pathogen cells on fresh produce surfaces than the dipping inoculation method (Beuchat et al., 2001). Initial inoculated populations of *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua* in cocktail were 8.34, 7.09, 5.72 and 7.17 log CFU/g on lettuce and 8.13, 7.65, 5.52 and 6.12 log CFU/g on strawberry respectively. When these bacteria were inoculated separately, the average numbers of *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua* were 8.33, 7.07, 5.84 and 7.54 log CFU/g on lettuce and 8.21, 7.39, 5.78 and 7.18 log CFU/g on strawberry respectively. The numbers of *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua* recovered from lettuce and strawberry samples were similar irrespectively the bacteria were inoculated separately or as a cocktail, which demonstrated that all bacteria retained similar attachment. The bacterial attachment is in accordance with other studies (Yang et al., 2003). Other studies have also selected cocktail inoculums due to their simultaneous prevalence of all these strains in vegetable and fruits (Bialka et al., 2008; Mahmoud, 2010; Ölmez and Temur, 2010; Sagong et al., 2011; Yaun et al., 2004).

In lettuce, treatments with UV reduced significantly the concentrations of all types of microorganisms (Fig. 1, $p < 0.05$). The reduction of four bacterial types depended on the different time intervals ($p < 0.05$) as well as on the bacterial type concerned. In lettuce, significant reduction of all bacteria was achieved after 20 min ($p < 0.05$). The reduction was finally about 1–1.7 logs in 45 min in all different types of microorganisms. Both *Salmonella* and *E. coli* in lettuce showed similar reductions when treated for the same period with UV, which is in accordance with the study of Yaun et al. (2004). In strawberries, the most significant reduction occurred after 10–30 min (1,2–3,6 J/cm^2) depending on the microorganism (Fig. 2, $p < 0.05$). Also, when UV dosages (7.56 kJ/m^2) have been used in other fruit surfaces (peaches and pears), better reductions of *E. coli* (up to 2.91 and 3.70 log CFU/g respectively) have been achieved (Syamaladevi et al., in press). In addition, reductions of *E. coli* and *Salmonella* in strawberries were achieved up to 2.5 log reduction, with dosages up to 64.4 J/cm^2 of pulsed UV light (Bialka et al., 2008).

The low log reduction recorded in our experiment was due to the UV lamp choice. The choice of a low dosage UV lamp technology was made in order to evaluate UV technology for disinfection taking into consideration that the food should not be affected in its color quality. In agreement to other findings (Allende and Artés, 2003; Bermúdez-Aguirre and Barbosa-Cánovas, 2013; Yaun et al., 2004), our work showed that

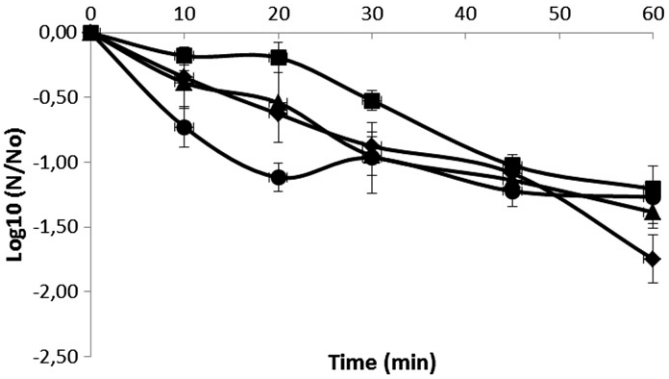

Fig. 1. Survival curves of *E. coli* (♦), *S. aureus* (■), *S.* Enteritidis (▲) and *L. innocua* (●) on lettuce exposed to UV for 10, 20, 30, 45 and 60 min.

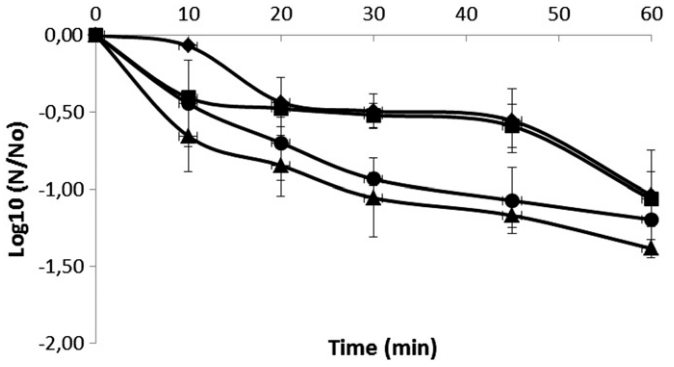

Fig. 2. Survival curves of *E. coli* (♦), *S. aureus* (■), *S.* Enteritidis (▲) and *L. innocua* (●) on strawberry exposed to UV for 10, 20, 30, 45 and 60 min.

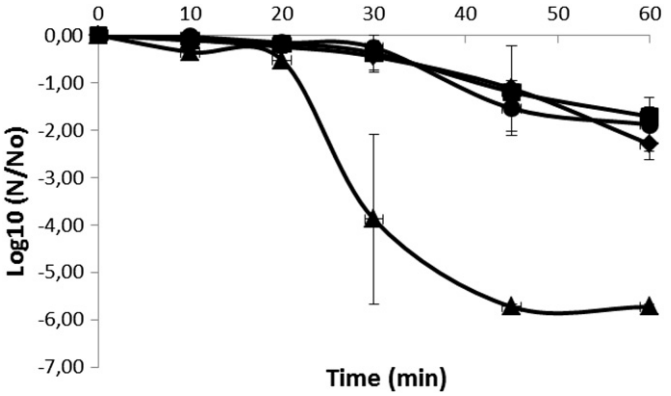

Fig. 3. Survival curves of *E. coli* (♦), *S. aureus* (■), *S.* Enteritidis (▲) and *L. innocua* (●) on lettuce exposed to ultrasound for 10, 20, 30, 45 and 60 min.

higher UV-C doses resulted in a greater decrease of bacterial growth in 'Romaine' lettuce and in strawberry pieces. The efficacy of surface disinfection by UV-C on food surfaces is influenced by several factors including: UV-C dose, UV-C dose rate, exposure time, surface characteristics, initial bacterial inoculum level and bacterial type (Otto et al., 2011). Despite the known limited ability of UV light to penetrate rough food surfaces, this study demonstrated that UV light has the potential to reduce bacterial contamination on food surfaces such as lettuce and strawberry surface and therefore has the potential to be used as post lethality treatment to control pathogens in ready to eat foods. However, UV-C was less effective at reducing populations of all bacterial types in strawberries when compared to lettuce. To predict UV disinfection rates on food surfaces, more kinetic inactivation data need to be obtained for pathogen and spoilage microorganisms, taking into account interactions between microorganisms and surface materials, such as shielding effects from incident UV and their dependency on surface structure or topography. Considering the bacteria inoculated into both lettuce and strawberry, more bacteria could have colonized deeper inside the strawberry, which could have reduced the chance of bacterial exposure to the UV-C light. Therefore the internalized bacteria in strawberry were possibly less affected by the UV-C light. It is known that UV-C light cannot penetrate deeply into the fresh produce (Morgan, 1989). To our knowledge, only two studies reported the use of UV-C to inactivate the internalized human pathogens in vegetables. A study conducted by Hadjok et al. (2008) showed that UV-C (37.8 mJ/cm^2) combined with 1.5% H_2O_2 continuous spraying at 50 °C achieved a 2.84 log reduction of the internalized *S.* Montevideo in iceberg lettuce. The second study of Ge et al. (2013) showed that UV-C irradiation with higher fluencies (150, 450, 900 mJ/cm^2) can significantly reduce the internalized *S.* Typhimurium in iceberg lettuce. However, the mechanism of UV-C against internalized bacteria in the plant has not been clearly illustrated and needs further exploration. Moreover, it has been shown that more irregular and complicated surfaces are less decontaminated (Luksiene et al., 2012). Since UV-C light has limited penetration and depth, plant morphological characteristics such as roughness and presence of wounds on fruit surfaces impact microbial inactivation. Understanding these influences are needed if this technology is to be commercialized (Schenk et al., 2008). However, limited information is available on the influence of fruit surface properties on the efficacy of UV-C for surface decontamination.

Treatment with US significantly reduced the numbers of all microorganisms on lettuce (Fig. 3, $p < 0.05$) and strawberries (Fig. 4, $p < 0.05$). The reduction of all four bacterial types in lettuce was significant after 30 min of treatment ($p < 0.05$). In strawberries, the reduction was significant after 30–45 min except for *Listeria* which was after 10 min ($p < 0.05$). The maximum reductions of *E. coli*, *S. aureus*, *S.* Enteritidis and *L. innocua* on lettuce were 2.30 ± 0.34, 1.71 ± 0.20, 5.72 ± 0.05 and 1.88 ± 0.57 log CFU/g observed, whereas in strawberries the reductions were 3.04 ± 0.72, 2.41 ± 0.59, 5.52 ± 0.13 and 6.12 ± 0.04, respectively (Fig. 4). Similar results were observed by other researchers (Sagong et al., 2011; São José and Dantas Vanetti., 2012). Reduction of microorganisms by US is mainly due to the physical phenomenon called cavitation (Alegria et al., 2009; Piyasena et al., 2003; Seymour et al., 2002).

Non-thermal technologies are being applied in food processing as an alternative to thermal processing. These green technologies can deliver food products without hazardous microorganisms and enzymes that may reduce the nutritional and sensory characteristics of foods, which are often changed when thermal processes are applied (Butz and Tauscher, 2002). Additionally, the use of chemical sanitizers has been associated with the formation of carcinogenic compounds in the last few years, and some pathogens have been shown to be more resistant to the lethal action of these compounds (Allende et al., 2008). There is a continuous need to provide fresh and microbiologically safe fresh-cut produce for consumers. Therefore, researchers are seeking new ways to reduce pathogens as well as chemical use to ensure the safety of fresh produce. Hence, the appropriate dose and treatment time of Ultrasound and Ultraviolet light methods used alone or in combination with other methods need to be developed. In this study, various UV and ultrasound treatments (different treatment times) were examined, to test their disinfection efficiency of different microorganisms in romaine lettuce and strawberry. However, the synergistic effect of the aforementioned methods with the use of heat, pressure and aqueous sanitizers was not performed in this study.

The log reduction of the bacterial cocktail compared in different foods and two disinfection methods is shown in Fig. 5. The results presented illustrate further the misconception that reductions of microorganisms on a food exhibit a log-linear trend (Bialka et al., 2008).

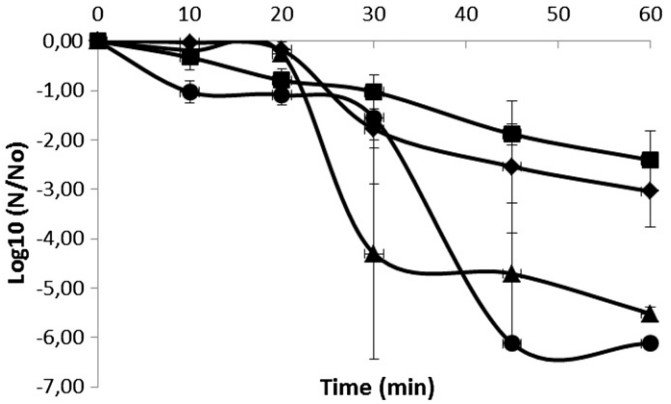

Fig. 4. Survival curves of *E. coli* (♦), *S. aureus* (■), *S.* Enteritidis (▲) and *L. innocua* (●) on strawberry exposed to ultrasound for 10, 20, 30, 45 and 60 min.

This was expected for both UV-C and US treatments since the intensity of both treatments was increasing with time exposure.

In addition, treatment with US or UV for <45 min did not significantly affect the general appearance and texture of lettuce and strawberry (data not shown). The leafy structure of lettuce showed an intense decrease of luminosity when ultrasound treatment was implemented and especially after 30 min of treatment. Samples that showed the highest net change of color (ΔE) were the samples treated with ultrasound at longest time intervals (45 and 60 min). When samples were treated with ultrasound for 45 and 60 min, some parts of the leafy structure lost their green color (a* −1.78, a* −3.42). The highest ΔE* of the treatments was when lettuce was treated with ultrasound for 45 min (ΔE*18.59) and for 60 min (ΔE*17.08). As far as strawberry modifications are concerned, C* value, which shows the degree of saturation, purity and intensity of color changed for the samples treated with UV for 45 min and 60 min as well as for samples treated with ultrasound at the same times, compared to the control sample.

Changes in color were measured and quantified in both foods after treatments with UV and US, in all time intervals. Results are shown in Table 1. The highest net change of color (ΔE) for lettuce was observed when the samples were treated with US at longest time intervals (Table 1), which indicated that a significant non-enzymatic browning reaction was present (Cao et al., 2010). Similar results were observed in another study for UV treated lettuce (Bermúdez-Aguirre and Barbosa-Cánovas, 2013). Strawberry color is a mix of red and yellow. Thus, Hunter a* and b* values or some combination of a* and b* may be considered as the physical parameters describing visual color degradation (Rodrigo et al., 2007). Both a* and b* values showed significant differences from 45 min of treatments ($p < 0.05$) with both disinfection methods (UV + US). It was obvious that C* value, which shows the degree of saturation, purity and intensity of color changed for the strawberry samples treated with UV for 45 min and 60 min as well as for strawberry samples treated with ultrasound at the same times, compared to the control sample. The color of the surrounding liquid was also changed after 60 min of Ultrasound treatment and a slightly pink color was obvious (data not shown). Moreover, the texture and the general appearance were modified after 45 min of Ultrasound treatment, whereas there was no obvious modification of the appearance after 45 min of UV treatment. The decrease of C* value may be attributed to enzymatic oxidation of anthocyanins leading to losses of color brilliancy (Holzwarth et al., 2012). In another study, thermosonicated strawberry samples retained their color (Alexandre et al., 2011). Aday et al. (2013) reported that changes in L* values in US treated strawberries were not significantly important. However, 90 W treatment had an adverse effect on the anthocyanins' stability. Moreover they reported that the

Table 1
Color parameters and color functions for lettuce and strawberry after disinfection with treatments of ultraviolet light and ultrasound.

Treatments	Color parameters				
	L*	a*	b*	ΔE*	C*
Lettuce					
Control	38.06 ± 0.19	−10.34 ± 0.57	20.28 ± 1.66		22.81
UV 10 min	35.81 ± 2.44	−6.54 ± 1.89	18.22 ± 4.41	6.58	19.36
UV 20 min	32.37 ± 0.43*	−5.83 ± 0.45	18.62 ± 0.23	7.67	19.51
UV 30 min	32.30 ± 0.14*	−7.59 ± 0.51	20.40 ± 0.85	6.37	21.77
UV 45 min	30.98 ± 0.15*	−5.43 ± 3.81*	17.40 ± 0.60	8.98	18.23
UV 60 min	29.52 ± 0.29*	−6.12 ± 0.97	17.50 ± 0.17	9.73	18.54
US 10 min	37.01 ± 0.17	−10.89 ± 1.75	22.99 ± 0.52	2.88	25.43
US 20 min	33.59 ± 2.27*	−13.69 ± 5.42	23.18 ± 3.14	5.51	26.93
US 30 min	28.72 ± 0.26*	−3.68 ± 0.36*	16.29 ± 0.26	11.98	16.70
US 45 min	24.39 ± 0.36*	−1.78 ± 1.27*	11.28 ± 2.30*	18.59	11.42
US 60 min	23.64 ± 0.29*	−3.42 ± 1.17*	13.40 ± 0.62*	17.08	13.83
Strawberry					
Control	21.96 ± 1.08	9.73 ± 1.26	13.16 ± 2.81		16.36
UV 10 min	21.25 ± 0.85	9.80 ± 4.35	14.57 ± 3.36	1.91	17.56
UV 20 min	20.62 ± 0.53	7.72 ± 3.80	12.79 ± 2.60	2.08	14.94
UV 30 min	17.37 ± 0.37	7.94 ± 2.23	10.60 ± 1.79	4.76	13.24
UV 45 min	15.89 ± 2.61*	6.74 ± 3.50	6.48 ± 2.47*	10.66	9.35
UV 60 min	15.70 ± 3.34*	5.09 ± 1.63	7.10 ± 0.75	11.69	8.73
US 10 min	20.40 ± 1.06	7.91 ± 0.80	15.32 ± 4.01	2.89	17.25
US 20 min	20.20 ± 0.97	6.11 ± 1.93	14.87 ± 2.13	4.12	16.08
US 30 min	18.17 ± 1.51	6.28 ± 1.49	12.40 ± 1.61	5.55	13.90
US 45 min	17.23 ± 2.73	4.46 ± 1.80*	11.21 ± 5.68	8.69	12.07
US 60 min	14.86 ± 5.08*	2.34 ± 3.24*	9.67 ± 7.60	14.29	9.95

Values are average ± standard deviation of at least three experiments and represent the color parameters after each processing time with each disinfection method: UV: Ultraviolet irradiation (254 nm), US: Ultrasound Treatment (Frequency: 37 kHz, Power: 30 W/L). Asterisks within different treatment methods indicate significant differences ($p < 0.05$).

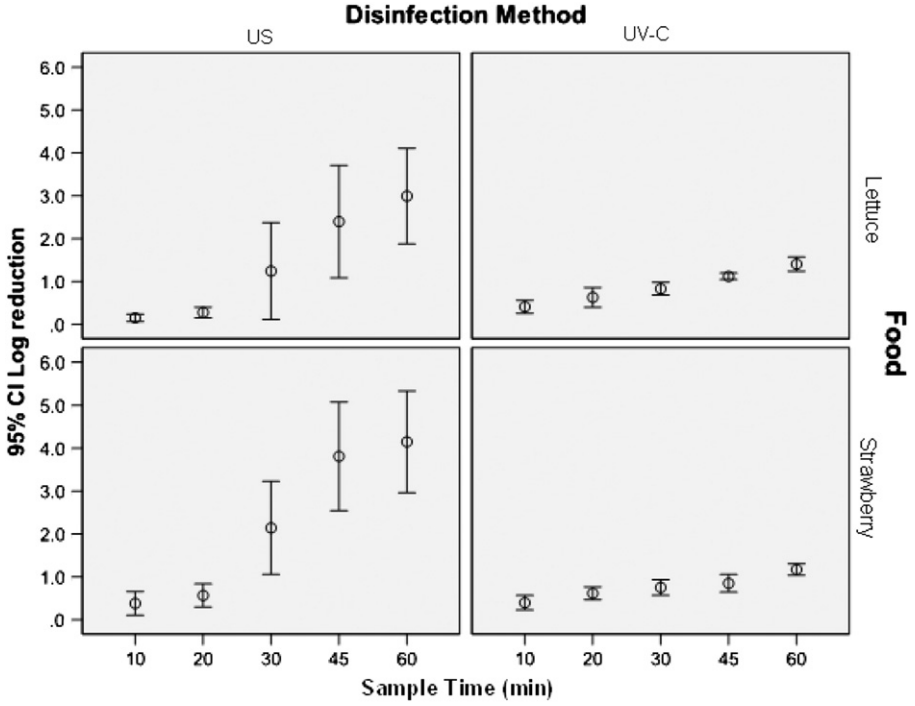

Fig. 5. Log reduction of each food type according the disinfection method used. Food 1: Lettuce; Food 2: Strawberry; Disinfection method 1: Ultrasound; Disinfection method 2: Ultraviolet.

bright color of strawberry was preserved when 30 W and 60 W treatments were implemented for 5 and 10 min. In our study, the treated samples with 30 W/L ultrasonication, retained their bright color (L*, a*, b*, C*) for treatments of 10, 20 and 30 min, which is in accordance with the study of Aday et al. (2013).

Many studies have been published, explaining the increase of use of alternative disinfection techniques, trying to totally replace the thermal ones in the industry (Alexandre et al., 2012; Allende and Artés, 2003; Bermúdez-Aguirre and Barbosa-Cánovas, 2013; Sagong et al., 2011; Syamaladevi et al., in press). However, a direct comparison between inactivation rates of a cocktail of microorganisms on different food surfaces using only non thermal techniques has not been studied yet by previous investigators. A direct comparison between the inactivation rates of four microorganisms, inoculated on two different produces, treated with UV and US was studied here.

When US were used, better disinfection efficacy was presented in strawberries than lettuce for the majority of microorganisms. This may be attributed to different food surface properties such as hydrophobicity, electric charge, and roughness that can influence the adhesion and distribution of bacterial cells on food surface (Araujo et al., 2010). On the other side, when UV was used, the disinfection rates were more or less the same between the two produces. The survival of microorganisms depends upon several other factors such as type of strain, initial inoculums level, surface characteristics, and growth conditions (Guerrero-Beltrán and Barbosa-Cánovas, 2004).

The objective of using cocktail experiments was to simulate real conditions that can occur during the food production chain of the lettuce and the strawberry, since all bacteria are considered important for the food industry. *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* are the main pathogens implicated in several foodborne outbreaks related to fresh produce (Baird-Parker, 1990; Francis et al., 1999; Griffin and Tauxe, 1991; Sagong et al., 2011). Moreover, *S. aureus* is important as it concerns the contamination of food by food handling (Lehto et al., 2011; Oliveira et al., 2011). There are only a few studies that examine the effect of non-thermal technologies in the disinfection of one or two pathogens (Alexandre et al., 2011; Bermúdez-Aguirre and Barbosa-Cánovas, 2013; Bialka et al., 2008; São José and Dantas Vanetti, 2012; Oliveira et al., 2011; Syamaladevi et al., in press; Yaun et al., 2004). Our study may give more realistic and valuable information for disinfection efficiency on different bacteria that can contaminate the fresh produce.

In conclusion, this is the first report that describes the inactivation of inoculated *E. coli*, *L. innocua*, *S.* Enteritidis and *S. aureus* on lettuce and strawberry by UV and US. This study demonstrated that, both treatments were effective against the tested bacteria and did not adversely affect food color. Both treatments could be good alternatives to other preservative techniques that are currently being used by the produce industry, due to their low cost, lack of extensive equipment and low energy consumption. The effectiveness of these two disinfection methods, were shown to be influenced by the dose, the exposure time and the surface of the food product. Some changes in the color of produce can be controlled if the exposure time is kept as low as possible, so as to inactivate effectively the microorganisms, but to still preserve the quality of the product. Therefore UV and US may be of benefit to those with little capital to invest as a means of ensuring product safety and quality. Thus, a large scale of experiment will be needed to determine the process conditions for industrial application.

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