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Bactericidal effect of ultraviolet-C treatments applied to honey

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ABSTRACT

Short wave ultraviolet light (UV-C) was studied in honey to inactivate vegetative cells of *Escherichia coli* (CECT 405) and spores of *Bacillus subtilis* (CECT 12) and *Clostridium sporogenes* (CECT 553) inoculated at a level of 10^4–10^5 CFU/g. UV-C doses ranging from 1.5 to 21.6 J/mL were used passing inoculated honey samples through an UV-C reactor up to 4 times. Lethal effect increased with both the final dose applied and number of passes through the UV-C reactor. *E. coli* was the most sensitive obtaining maximum reductions above 5 Log_{10} CFU/g at 14.4 J/mL in treatments with 2 passes while for *B. subtilis* spore reductions of just 2.7 Log_{10} CFU/g were obtained after the same treatment. For spores of *Cl. sporogenes* maximum reduction of 2.5 Log_{10} CFU/g was observed after an 18 J/mL treatment. No significant differences ($P > 0.05$) were observed when treatments were applied with three passes or less, but after 4 passes, spore reduction above 3.5 Log_{10} CFU/g was achieved. Effect of UV-C on some quality parameters of honey, such as hydroxymethylfurfural, pH and color, was also assessed. UV-C light made changes in most of these parameters although this not necessarily implied a reduction in the quality of honey.

1. Introduction

Honey is a natural food produced by *Apis mellifera* by collecting the nectar of plants, secretions of living parts of plants or secretions of plant-sucking insects and composed of different sugars, predominantly fructose and glucose. Differences in flavor and aroma are determined by the floral origin, the climate and the environmental conditions (Karimov, Xalilzad, Hobbi, & Alekperov, 2014; Różańska, 2011; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Pérez-Álvarez, 2008). Honey is usually considered a microbiological safe food, with a long shelf life, not requiring refrigeration, due to its low pH and reduced water activity. Therefore, only a limited number of microorganisms are expected to survive and eventually grow in honey, mostly yeasts and molds, as well as some spore forming bacteria whose sources are the pollen and nectar collected by bees, dust, air or the handling during collecting and processing of honey (Gomes, Dias, Moreira, Rodrigues, & Estevinho, 2010; Grigoryan, 2016; Iurlina & Fritz, 2005; Różańska, 2011; Snowdon & Cliver, 1996).

Infant botulism is a disease that affects children under the age of one year that is induced by the ingestion of food contaminated by spores of *Cl. botulinum*, that can colonize and germinate in the immature intestinal tract of infants synthesizing the botulinic neurotoxin, which is absorbed into the bloodstream and carried to peripheral cholinergic synapses, where the toxin binds irreversibly. This neurotoxin blocks the release of acetylcholine at somatic and autonomic nerve terminals (Abe, Negasawa, Monma, & Oka, 2008; Arnon & Midura, 1977; Fox, Keet, & Strober, 2005; Lawrence, 1979; Rudnicka, Kwiatkowska, Gajewski, & Chmiela, 2015; Sobel, 2005). Infant botulism is rare, but is the most common form of botulism in the United States, where about 90% of the cases worldwide are reported (Rosow & Strober, 2015). The minimal infective dose for infants has been estimated between 10 and 100 spores. As honey is the food most usually associated with infant botulism it is usually recommended to avoid feeding infants less than 1 year with honey (Gill, 1982; Radšel, Andlovic, Neubauer, & Osredkar, 2013). Heat treatments would increase the safety of honey to make it suitable for infants consumption (Grigoryan, 2016), but honey contains a large number of thermosensitive compounds responsible for its flavor and for some of its functional properties. The loss of these minor compounds is proportional to the temperature and the duration of thermal treatments and may cause an alteration of the essential composition, and consequently is not accepted as a current practice (Lado & Yousef, 2002; Tosi, Ré, Lucero, & Bulacio, 2004).

UV light in the range of 200–280 nm (UV-C) is lethal to most microorganisms, including bacteria, viruses, fungi and yeast, but the efficiency differs among species, strains and even growth stage of the culture. UV-C causes a rearrangement of the nucleic acids of microorganisms affecting its reproducibility (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Keyser, Muller, Cilliers, Nel, & Gouws, 2008; Salas-

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Vicente et al., 2016). UV-C treatments have been proposed as a simple and environmentally friendly way to eliminate pathogen and spoilage microorganisms from foods such as fruit juices, wine and dairy products (Bintsis et al., 2000; Keyser et al., 2008; Fredericks, du Toit, & Krügel, 2011). The bactericidal action of the treatment depends primarily on the dose of UV light applied to the microorganism that is cumulative over time (Fredericks et al., 2011), but matrix characteristics may affect the absorbance of UV-C light causing different efficiencies of the irradiation (Koutchma, Keller, Chirtel, & Parisi, 2004).

The aim of this work was to investigate the feasibility of UV-C light treatments to decontaminate honey in order to increase its safety. With this aim, different microorganisms were selected as markers of processing contamination, such as *Escherichia coli*, spores of *Bacillus subtilis* and *Clostridium sporogenes*. The later has been proposed in different surveys as surrogate for *Clostridium botulinum* because it belongs to a close related phylogenetic unit and comprises similar characteristics of the target pathogen strain except the toxicity (Bradshaw et al., 2010; Collins & East, 1998).

2. Materials & methods

2.1. Preparation of bacterial cultures

Escherichia coli (CECT 405), *Bacillus subtilis* (CECT 12) and *Clostridium sporogenes* (CECT 553) strains were obtained from the Spanish Type Culture Collection (CECT, University of Valencia, Spain) as lyophilized cultures. *E. coli* was rehydrated with 3 mL of Trypticase-Soya Broth (TSB, Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h. The resulting culture was streaked on a Petri plate containing Tryptic Soy Agar (TSA, Oxoid) and incubated at the same time and temperature. After checking the purity of the culture one colony was streaked in a tube containing TSA and incubated at 37 °C for 24 h. This culture was kept at 4 °C to preserve the freshness of the culture until being used.

Spores of *Bacillus subtilis* and *Clostridium sporogenes* were obtained according to the procedure described in the EN/ISO 13704 norm European Standard 13704 (2002). For *Bacillus subtilis* 1.0 mL of the rehydrated lyophilized culture was transferred to a tube containing 10 mL of Tryptone Glucose Broth (TGB, Oxoid) and incubated at 30 °C for 24 h. Subsequently 0.5 mL of this culture was transferred to each of several Roux flasks containing Malt and Yeast Extract Agar (MYA, Oxoid) and incubated at 30 °C for 8–10 days. The resulting culture was then suspended in 15 mL of sterile distilled water using a sterile loop. All the fractions of spore suspension were collected in a Falcon flask and purified centrifuging it at 10,000 *g* for 20 min at 4 °C. The resulting pellets were suspended with 30–40 mL of sterile distilled water. This procedure was repeated four times. The final pellet was suspended in 30 mL of distilled water, subjected to a thermal shock at 75 °C for 10 min and cooled placing the tubes in a bath of water and ice. The resulting suspension was kept at 4 °C until being used.

For *Clostridium sporogenes*, original culture was rehydrated with 4.0 mL of Triptone Water (TW, Oxoid) and transferred to tubes (1.0 mL each) with 9.0 mL of sterilized TW sealed with semi-liquid sterile Vaseline and incubated at 37 °C for 48 h. The obtained culture was transferred to a culture Roux flask containing Triptone Agar (TA, Oxoid) and incubated under an anaerobic atmosphere for 15 days at 37 °C. Spores were then suspended in 35 mL of sterile distilled water and centrifuged at 10,000 *g* for 20 min at 4 °C. This procedure was repeated at least two times. The resulting suspension was sonicated with a Branson E-MT Ultrasonic Cleaner (Branson Ultrasonics Corp., Dambury, CT, USA) for 5 min to release spores from mother cells, and later incubated at 37 °C for 2 h. Two additional centrifugation procedures were then applied at 2050 *g* for 20 min at 20 °C. The resulting pellet was then suspended in 35 mL of sterile distilled water submitted to a heat shock at 80 °C for 15 min, cooled placing the tubes in a bath of water and ice and kept at 4 °C until being used.

2.2. Inoculation of honey samples

Multiflora bee honey of commercial origin (absorption coefficient of 30/cm measured at 254 nm) was first tempered at 45 °C in a water bath in order to promote its fluency and ensure a good and uniform distribution of the inoculates. For each UV-C treatment 1 kg of honey was inoculated with 10 mL of bacterial suspensions leading to a final concentration of about 10^5 CFU/g in the case of *Escherichia coli* and *Bacillus subtilis,* and 10^4 CFU/g in the case of *Clostridium sporogenes*. Due to the high viscosity of honey, and with the aim to ensure maximum homogeneity of the inoculum, the amount of honey necessary for each treatment was divided into smaller fractions to which the proportional part of the inoculum was added and mixed thoroughly with a sterile spatula. After this process, the different fractions were combined and an additional mixture was made. All these actions were carried out inside a biosecurity cabinet (model Bio II-A, Telstar, Terrassa, Spain) to avoid any risk of environmental contamination of the sample.

2.3. UV-C treatments

The UV-C equipment used for the treatments is composed of a tank of 2.0 L capacity, a peristaltic pump Flowmaster FMT300 (ISMATEC Lab. GmbH, Wertheim-Mondfeld, Germany) that feeds the circuit, and a UV-C reactor (UV-Therm, Ypsicon, SL, Barcelona, Spain) formed by a UV-C lamp with a power of 31 mW/cm^2 at 254 nm, protected with a concentric quartz tube. The reactor has a capacity of about 70 mL and samples pass through it with a thickness of 1 mm. The flow can be regulated between 3.0 and 90 L/h in order to adjust the time to receive the necessary UV-C dose. Table 1 shows the equivalences between time of exposure (in seconds) and received UV-C doses expressed in different units. Temperature of the sample was adjusted to 45 °C using a heat conditioner placed at the entrance and along the UV-C reactor.

2.4. Microbiological analysis of UV-C treated samples

E. coli and *B. subtilis* counts were obtained from plates of TSA agar supplemented with a 0.6% of yeast extract (Oxoid) (TSAYE) incubated at 30 °C for 48 h. Counts of *Cl. sporogenes* were obtained from plates of Glucose-Meat-Yeast Agar (MGYA) prepared according European Standard 13704 (2002) after incubating for 48 h at 37 °C under anaerobic conditions.

Lethality caused by the UV-C treatments was calculated according to the following equation:

$$Lethality = \log_{10}\left(\frac{N_0}{N+1}\right)$$

Where N_0 is the initial number of CFU/g before the UV-C treatment (control) and N expresses the number of CFU/g after the UV-C treatment.

Table 1
Equivalences of UV-C treatment times and UV-C doses expressed in different units using a concentric tubular reactor with an inner volume of 70 mL and a 31 mW/cm^2 lamp.

Time (sec)	J/cm^2	J/mL	J/L
1	0,03	0,3	298
10	0,31	3,0	2980
12	0,37	3,6	3576
15	0,47	4,5	4470
20	0,62	6,0	5960
24	0,74	7,2	7152
30	0,93	9,0	8940
40	1,24	12,0	11920
45	1,40	13,5	13410
48	1,49	14,4	14304
60	1,86	18,0	17880
72	2,23	21,6	21456
120	3,72	36,0	35760

2.5. Physicochemical analysis of honey

2.5.1. Hydroxymethylfurfural (HMF)

For the determination of the HMF the method AOAC (2000) was used, where 5 g of honey was dissolved in 25 mL of distillated water and transferred to a 50 mL calibrated volumetric flask with 0.5 mL of Carrez I and 0.5 mL of Carrez II reagents, adjusting the final volume to 50 mL with distilled water. This solution was then filtrated (Whatman 1) and 5 mL of the filtrate was mixed with 5 mL of sodium bisulfite (0.2%) (Panreac AppliChem, Barcelona, Spain). Absorbance was measured at 284 nm and 336 nm using an Ultrospec 2100 pro spectrophotometer (Amersham Biosciences Corp., Piscataway, USA) and HMF content calculated using the formula:

$$\text{HMF (mg/100 g)} = ((A1\text{-}A2)*f*5)/P$$

Where A1 is the absorbance at 284 nm, A2 the absorbance at 336 nm, P the weight of the sample (g) and "f" (= 14.97) the necessary factor to express the HMF in mg/100 g.

2.5.2. Color

Color parameters ($L+$, a^* and b^*) were measured with a HunterLab MiniScanTM XE (Hunter Associates Laboratory Inc., Reston, VA). To evaluate the samples as a uniform color space, the total color difference (ΔE) from each sample was calculated applying the formula:

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$$

2.5.3. pH

The pH was determined according to Vanhanen, Emmertz, and Savage (2011) with a pH-meter micro pH 2000 (CRISON Instruments SA, Alella, Barcelona, Spain) placing the electrode directly on a 20% (w/v) solution of the honey with distilled water to facilitate the measurement without affecting the pH value.

2.6. Statistical analysis

All the experiments were repeated a minimum of three times, and samples from each treatment were analyzed in duplicate. Results were compared using ANOVA of one factor and Tukey test. Level of significance was set at $P < 0.05$. The statistical analysis was performed with the program IBM SPSS statistics 24 (IBM software, Somers, New York, USA, 2012).

3. Results

3.1. Effect of UV-C on E. coli

Fig. 1 shows the effect of UV-C treatments on the viability of vegetative cells of *E. coli* inoculated in honey. The lethality achieved increased with the time of exposure to the UV-C and consequently with the dose received. Maximum lethality (5.37 Log_{10} CFU/g) was obtained at a dose of 21.6 J/mL (72 s of exposure) that was at the limit of determination considering the initial load of *E. coli* in honey, but when samples were passed two times through the reactor lethality was maximum after just 48 s of exposure (14.4 J/mL). When these results were adjusted to a 1st order function R^2 values above 0.90 were obtained in both cases (single pass and recirculation), but as some results were at the limit of determination adjust would not be accurate enough to be used as a predictive model.

3.2. Effect of UV-C on B. subtilis

Fig. 2 shows the effect of the UV-C treatments on *Bacillus subtilis* spores. As observed for *E. coli*, treatments with recirculation were more effective, achieving reductions of 3.57 Log_{10} CFU/g after an UV-C dose of 21.6 J/mL, compared with the 2.07 Log_{10} CFU/g reduction achieved after a single pass at the same dose. Lethality data adjusted very well to a first order function, with R^2 values of 0.9873 for a single pass and 0.9925 for recirculation. The slope was also higher when treatments were applied by recirculating the sample. In that case, the maximum lethality values achieved were below the limit making the adjustment more accurate. This allowed estimating a D value for *B. subtilis* described as the necessary UV-C dose (in J/mL) to reduce 90% of the load of *B. subtilis* spores in honey. This D value was estimated as 13.43 J/mL when treatments were applied in a single pass, and decreased to 7.42 J/mL when treatments were applied in two passes.

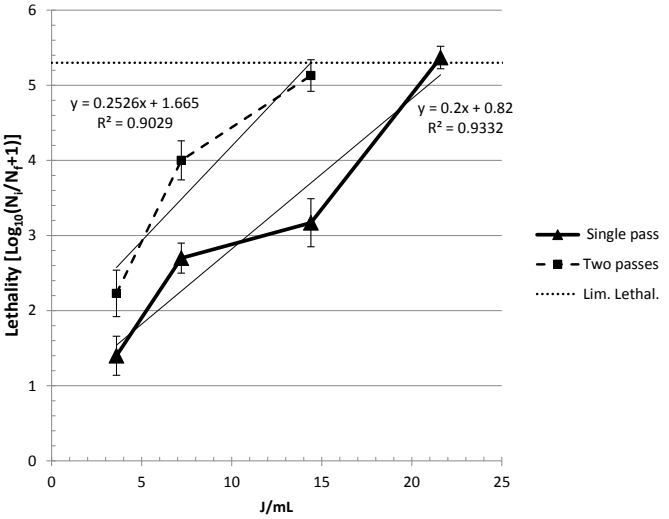

Fig. 1. Lethality values for *Escherichia coli* (CECT 405) in honey after UV-C treatments at 254 nm applied with a single pass and two passes throughout the UV-C reactor. Graphic shows mean values ± SD, as well as the line of tendency when values are adjusted to a first order function.

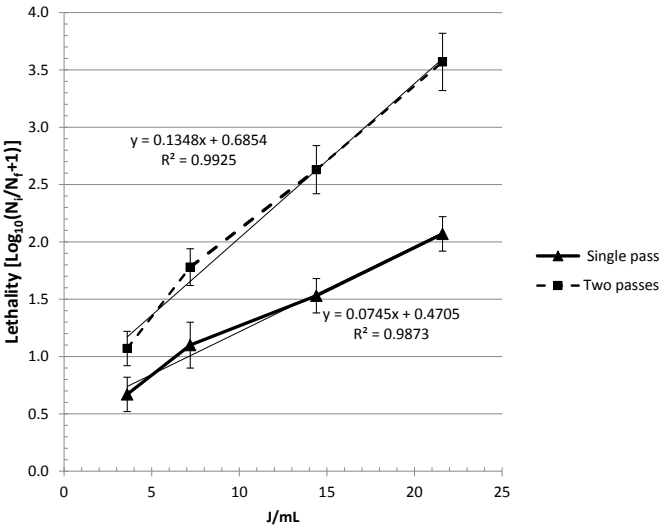

Fig. 2. Lethality values for *Bacillus subtilis* (CECT 12) in honey after UV-C treatments at 254 nm applied with a single pass and two passes throughout the UV-C reactor. Graphic shows mean values ± SD, as well as the line of tendency when values are adjusted to a first order function.

3.3. Effect of UV-C on Cl. sporogenes

Lethal effect of UV-C on *Cl. sporogenes* spores also increased linearly with the UV-C dose (Fig. 3), but significant differences between treatments were evidenced only when exposure to UV-C light was increased from 7.2 to 18 J/mL (in a single pass), increasing the lethality from 1.5

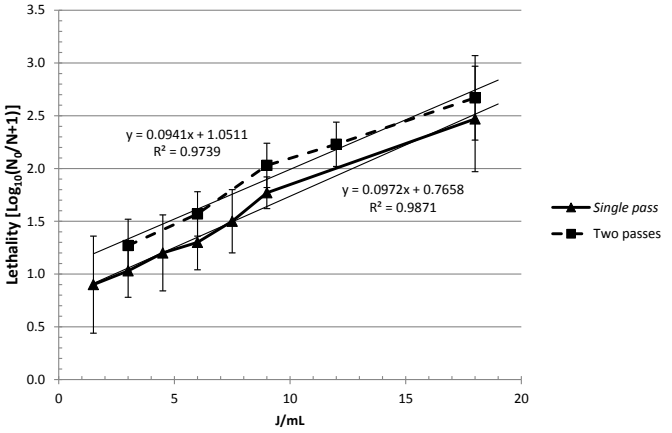

Fig. 3. Lethality values for *Clostridium sporogenes* (CECT 553) in honey after UV-C treatments at 254 nm applied with a single pass and two passes throughout the UV-C reactor. Graphic shows mean values ± SD, as well as the line of tendency when values are adjusted to a first order function.

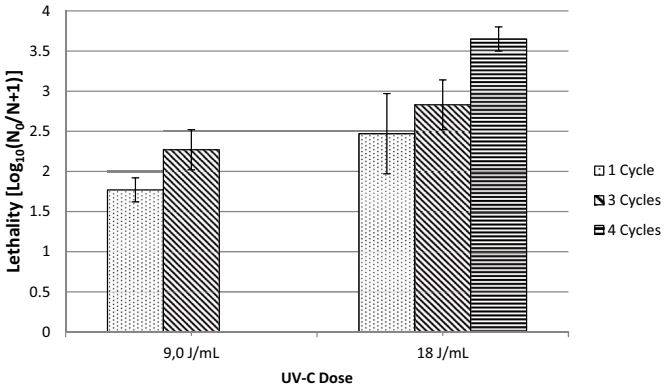

Fig. 4. Lethality values for *Clostridium sporogenes* (CECT 553) in honey after UV-C treatments of 9,0 and 18 J/mL applied with up to 4 passes throughout the UV-C reactor. Graphic shows mean values ± SD.

Log_{10} CFU/g to 2.5 Log_{10} CFU/g. Passing samples two times through the reactor did not increase significantly the efficiency of the treatment. When data were adjusted to a first order function, these were very similar for samples submitted to a single pass and recirculated, and consequently the estimated D-values were also very similar in both cases (10.53 and 10.63 J/mL, respectively). *Cl. sporogenes* showed to be more sensitive than *B. subtilis* when a single pass treatment was applied, since a dose of 13.35 J/mL would be sufficient to achieve the same reduction obtained for *B. subtilis* after a dose of 21.6 J/mL (2.07 Log_{10} CFU/g). However, this was not the case when the treatment was applied in to passes; according to the estimated D value, when two passes are used for the treatment, a dose of 26.76 J/mL would be needed for *Cl. sporogenes* to achieve the same lethality observed for *B. subtilis* after a UV-C dose of 21.6 J/mL. The effect of passing the samples three or four passes were tested for 9.0 and 18 J/mL UV-C doses (Fig. 4). After three passes mean lethality values increased at both doses tested, although this was only significantly higher after a 9.0 J/mL treatment. In the case of 18 J/mL four passes were necessary to increase significantly the lethality to a mean value of 3.65 Log_{10} CFU/g, 1.18 Log_{10} CFU/g higher than when a single pass was used with the same dose.

3.4. Effect of UV-C on physicochemical parameters

To measure the effect of UV-C on the studied physicochemical parameters of honey passes of 15 s through the UV-C reactor were used for technical reasons, ranging the resulting exposures from 15 s (4.5 J/mL) to a maximum of 120 s (36 J/mL). Table 2 shows the effect of these UV-C treatments, as well as the effect of remaining 120 s at 45 °C, on the evaluated parameters of honey. UV-C treatments caused a significant reduction on HMF concentration, decreasing from the initial value of 34.67 mg/kg to 9.90 mg/kg after receiving a dose of 36 J/mL. On the contrary, remaining 120 s at 45 °C incremented slightly the HMF concentration to 36.10 mg/kg, although not significantly. Table 2 also shows that the ΔE value (that compare the E values of the honey before and after the UV-C treatments) increased with the exposure to UV-C. For all samples ΔE values were higher than 5, which is considered a difference easily noticeable for the human eye (Stokes, Fairchild, & Berns, 1992). Nevertheless part of this change was due to the temperature that increased significantly the ΔE by itself. Mean pH value of non-treated honey was 4.16. UV-C treatments increased significantly this value, although this increase was lower than 0.06 pH units. In that case temperature did not influence significantly.

Table 2
Mean value ± SD of pH, HMF and color values of honey after UV-C treatments. Values of the same column with different superscript differ significantly (P < 0,05).

Treatments	pH	HMF (mg/100 g)	Color [ΔE]
0,0 (control)	4,16 ± 0,01 ^{a}	34,67 ± 0,94 ^{a}	–
4,5 J/mL	4,17 ± 0,02 ^{ab}	30,32 ± 0,63 ^{ab}	5,14 ± 0,53 ^{a}
9,0 J/mL	4,18 ± 0,02 ^{abc}	24,24 ± 3,52 ^{bc}	5,48 ± 1,11 ^{a}
13,5 J/mL	4,19 ± 0,02 ^{abc}	22,47 ± 2,22 ^{bc}	8,30 ± 0,82 ^{bc}
18,0 J/mL	4,20 ± 0,02 ^{bc}	17,46 ± 2,31 ^{cd}	7,31 ± 0,40 ^{b}
22,5 J/mL	4,19 ± 0,01 ^{bc}	14,55 ± 3,74 ^{d}	9,45 ± 0,15 ^{cd}
27,0 J/mL	4,20 ± 0,01 ^{bc}	14,48 ± 1,50 ^{d}	10,09 ± 0,45 ^{de}
31,5 J/mL	4,21 ± 0,01 ^{bc}	12,95 ± 3,31 ^{d}	11,08 ± 0,33 ^{e}
36,0 J/mL	4,21 ± 0,01 ^{c}	9,90 ± 3,37 ^{d}	14,03 ± 0,48 ^{f}
120 s 45 °C	4,15 ± 0,01 ^{a}	36,10 ± 3,35 ^{a}	7,49 ± 0,34 ^{b}

4. Discussion

UV-C treatments applied in a single pass were more efficient in *Escherichia coli* than in the spore-forming bacteria, achieving reductions of up to 5 Log_{10} CFU/g at 14.4 J/mL. No previous references were found about the effect of UV-C light on *E. coli* in honey, but several were found concerning other matrices. Koutchma et al. (2004), reported reductions above 5 Log_{10} CFU/mL on *E. coli* K-12 strain in apple juice and cider that depended on the color and absorbance coefficient (greater in clarified than in non-clarified juices). Keyser et al. (2008) also reported similar reduction in clarified apple juice at UV-C doses from 0.230 J/mL. Unluturk, Atilgan, Handan, and Tari (2008) investigated the reduction of *E. coli* in liquid egg white, observing a lethality up to 2 Log_{10} CFU/mL at doses below 30 mJ/cm^2. Crook, Rossitto, Parko, Koutchma, and Cullor (2015) determined that a dose of 1.5 J/mL was required to achieve a 5 Log reduction of *E. coli* O157:H7 in UHT whole milk. A dose of 3.6 J/mL was needed in this work to obtain a reduction of only 1 Log_{10} CFU/g of the initial *E. coli* loading and a 21.6 J/mL dose to achieve a reduction greater than 5 Log when a single pass treatment was applied. When the treatment was applied through two passes the required dose to achieve the same goal was only 14.6 J/mL. Concerning *Bacillus subtilis*, Chang et al. (1985) described that a dose of 36 mJ/cm^2 was required to reduce 90% (1 Log10 CFU/mL) the initial load of *B. subtilis* inoculated in waste water. Hijnen, Beerendonk, and Medema (2006) determined that the required dose in drinking water was just 16.9 mJ/cm^2. In this work it was needed a dose of 3.6 J/mL (372 mJ/cm^2) to achieve a similar reduction.

These differences could be attributed to both the differences in the equipment used to apply the UV-C treatments and to the physical properties of the matrices (e.g. absorbance coefficient) that may influence the UV-C effectiveness. Honey has a high optical density due to the presence of solids, solutes and organic compounds that leads to a high absorbance of light and light scattering, and this can greatly influence the transmission and absorbance of UV light, and consequently the

effect on the microorganisms (Koutchma, Forney, & Moraru, 2009). When a fluid flows in a laminar regime throughout the UV-C reactor it flows in the form of concentric layers and the UV-C radiation affects mainly the layers that are closer to the radiation source. For that reason a turbulent flow is recommended to increase the effectiveness of treatments as is reported in most of the previously mentioned references. In our reactor honey flowed in a layer of just 1 mm of thickness. This reduced thickness ensures that UV-C light can reach most of the sample flowing through the reactor when the matrix has a low absorbance coefficient, but the high viscosity of the honey causes it to flow in a laminar regime. As a consequence, some microorganisms could be protected from UV-C radiation because they were protected by particles (shadow effect), or by the high absorption coefficient of honey. Introducing different cycles that cause honey to be mixed between passes avoided the creation of permanent shadows and increased the effectiveness of the treatments.

Hillegas and Demirci (2003) is the only reference found concerning the application of UV-C to preserve honey. In this survey the effect of UV-C applied as pulses was evaluated on *Clostridium sporogenes*, observing that the degree of inactivation increased with the number of pulses and reducing the distance between the product and the lamp, and also the depth of honey, but reductions achieved were always below 1.0 Log_{10} CFU/g of the initial load. Nakano, Okabe, Hashimoto, and Sakaguchi (1989) detected a maximum of 60 spores of *Cl. botulinum* per gram in the 8.5% of samples of honey of different origins. Snowdon and Cliver (1996) consider that, if present, honey contains less than 1.0 CFU/g of *Cl. Botulinum*, however this amount may increase when honey contains higher amounts of beeswax, pollen or dust (Nevas, Lindström, Hörman, Keto-Timonen, & Korkeala, 2006). The minimal infection dose of *Cl. botulinum* for infants has been estimated between 10 and 100 spores (Gill, 1982; Radšel et al., 2013). Considering this a reduction above 3.0 Log_{10} CFU/g in the spore concentration could be enough to consider honey safe for children. Although counts of *Cl. sporogenes* spores decreased with the UV-C dose received, differences observed between doses were lower than with *E. coli* and *B. subtilis*, and lethality achieved was below the minimum required to guarantee a fair reduction of *Cl. botulinum* spores in honey. Nevertheless, increasing the number of passes allowed to increase significantly the lethality to a point of achieving the goal of at least 3.0 Log_{10} CFU/g reduction when four passes were used.

Concerning the effect of UV-C treatments on the physicochemical quality parameters evaluated, although pH values changed slightly due to UV-C, they were always maintained between the normal ranges of 3.42–6.10 according to FAO (1996). The color of honey has no limits on the EU regulations (Council Directive 2001/110/EC, 2002), but for all treatments ΔE was higher than 5, which is considered a change easily noticeable for the human eye (Stokes et al., 1992). However, natural color of honey is variable and depends mainly on its floral origin, thus these differences should not necessarily affect consumers acceptance. HMF is a quality control parameter used to evaluate damages caused by heating or inappropriate storage of honey (Sanz, Del Castillo, Corzo, & Olano, 2003). de Oliveira et al. (2012) observed that HMF content increases slowly when the temperature is below 70 °C, but above 80 °C HMF increases in a significant way. European Union regulations (Council Directive 2001/110/EC, 2002) established that HMF content should not be higher than 40 mg/kg. In this work the UV-C treatment caused a reduction of the HMF content that was linearly proportional to the UV-C dose received (R^2 of 0.94). No previous references were found concerning the effect of UV-C on quality parameters of honey, but Bera, Almeida-Muradian, and Sabato (2009) also reported a decreasing effect on HMF when honey samples of different origin were submitted to gamma irradiation (10 kGy). Considering these results, HMF is not a good parameter to estimate a quality reduction of honey when it is submitted to UV-C as it is for thermal treatments or inadequate storage.

5. Conclusion

From the results of this work, especially those concerning *Cl. sporogenes*, UV-C treatments could be considered a feasible alternative to heat treatments to guarantee the safety of honey and its aptitude for all kind of consumers. Nevertheless, it would be advisable to test these treatments directly on spores of different *Cl. botulinum* strains, as well as to evaluate the effect of UV-C on a wider range of quality parameters and kinds of honey to confirm this aptitude.

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