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Inactivation kinetics of foodborne pathogens by UV-C radiation and its subsequent growth in fresh-cut kailan-hybrid broccoli

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ABSTRACT

The inactivation of *Escherichia coli*, *S.* Enteritidis and *Listeria monocytogenes* after UV-C radiation with 0, 2.5, 5, 7.5, 10 and 15 kJ UV-C m^{-2} on fresh-cut kailan-hybrid broccoli was explored. Inactivation did not follow linear kinetics. Hence, it was modelled by using the Weibull distribution function, obtaining adjusted R^2 values higher than 94%, indicative of the accuracy of the model to the experimental data. The UV-C doses needed to reduce 1 log cycle the *E. coli*, *S.* Enteritidis and *L. monocytogenes* counts were 1.07, 0.02 and 9.26 kJ m^{-2}, respectively, being *S.* Enteritidis the most sensitive microorganism to UV-C radiation while *L. monocytogenes* was the most resistant. According to experimental data, UV-C doses higher than 2.5 kJ m^{-2} did not achieve great microbial reductions. No differences in the growth behaviour of these microorganisms was observed in the treated samples stored under air conditions at 5, 10 and 15 °C, compared to the control. Conclusively, low UV-C doses are effective to reduce *E. coli*, *S.* Enteritidis and *L. monocytogenes* populations in fresh-cut kailan-hybrid broccoli keeping such counts stable during shelf life at 5–10 °C. The current study provides inactivation models for these foodborne pathogens that can be used in microbial risk assessment.

1. Introduction

The consumer's increasing interest in broccoli is mainly due to its considerable relevance as a health-promoting fresh produce (Jeffery et al., 2003; Lemoine et al., 2010; Martínez-Hernández et al., 2013a; Podşedek, 2007). Kailan-hybrid broccoli is a natural hybrid between kailan (*Brassica oleracea*, Alboglabra group), and conventional broccoli (*B. oleracea*, Italica group). This morphologically distinct kailan-hybrid has a single floret with a long slender stem which may avoid the shading effect of conventional broccoli varieties. This particular broccoli hybrid has a mild sweeter taste than conventional broccoli varieties, being completely edible (both as whole raw and after soft cooking methods), and quite well adapted for fresh-cut purposes (Martínez-Hernández et al., 2013b).

In the last 20 years, the fresh-cut vegetables have gained more importance due to consumers' demand for fresh, convenient, preservative-free foods that may promote health (Artés et al., 2009). The growing demand of fresh-cut products raises the need for increased shelf-life and safety of these products. Fresh-cut products can be a vehicle for the transmission of foodborne pathogens since processing of these products implies removing of their natural barriers favouring possible pathogen contamination. Accordingly, several outbreaks in fresh-cut products have been reported in the last years (Olaimat and Holley, 2012). The major concerns are with enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella* spp that have fast growth rates and low infectious doses (Martin, 2007). Furthermore, listeriosis remains a great public health concern, as it has one of the highest case fatality rates of all the foodborne infections in Europe with 20–30% (Martin, 2007). Accordingly, the microbial limits stipulated for fresh-cut products by the European Regulation EC 1441/2007 (2007) are limited to *E. coli*, *Salmonella* spp and *Listeria monocytogenes*. In that way, estimation of pathogen growth and inactivation kinetics has great interest in the context of quantitative risk assessment.

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Washing with chlorine (sodium hypochlorite solution, 50–200 mg L^{-1} for 1–2 min at pH approx 6.5) is a widely used sanitising method by the fresh-cut industry. However, it may be potentially harmful for humans and the environment. Thus, several alternative techniques such as UV-C radiation were suggested to preserve quality of horticultural products (Artés et al., 2009; Allende et al., 2006). In the last decade several works have studied the effects of UV-C radiation on inoculated fresh-cut products (lettuce, tomatoes, mushrooms, clover spouts, baby spinach, jalapeño pepper, apples, blueberries, etc.) with *E. coli*, *Salmonella* spp and *L. monocytogenes* as it is reviewed in Table 1. However, the effectiveness of UV-C irradiation may depend on several factors such as those related to the conditions of the produce inoculation and the UV-C instrument parameters (Kim et al., 2013). This makes it difficult to interrelate UV-C effectiveness on inoculated pathogens between published works (as reviewed in Table 1) since different conditions were used.

Attending to surface or internalized pathogen inoculation of produce, it has been recently reviewed that pathogens internalized appear to have short-term persistence (Erickson, 2012). Accordingly, as observed in Table 1 Hadjok et al. (2008) found that a UV-C treatment of 0.378 kJ m^{-2} applied to iceberg lettuce internally-inoculated (using vacuum system) with *Salmonella* Montevideo P2 did not achieve significant pathogen reduction while the same UV-C dose achieved a 2-log CFU g^{-1} reduction on the surface-inoculated lettuce. However, Ge et al. (2013) found that UV-C irradiation (0.75–9 kJ m^{-2}) significantly reduced in 1.96–2.52 log units *S. Typhimurium* internalized in iceberg lettuce while chlorine washing (up to 200 mg L^{-1} for 10 min) or peroxyacetic acid (up to 80 mg L^{-1} for 10 min) were not effective.

Surface inoculation is generally conducted by either submersion (3 s-20 min) or spot inoculation followed by an air-drying process (Table 1). Higher reductions of *L. monocytogenes* inoculated by submersion have been reported in fresh-cut lettuce after UV-C treatments compared to the same microorganism spot-inoculated in fresh-cut salad (Chun et al., 2010; Kim et al., 2013). Similarly, higher Salmonella spp. reductions after UV-C treatments have been found in tomatoes inoculated by submersion compared to those inoculated with the same microorganism by spot inoculation (Sommers et al., 2010; Yaun et al., 2004). However, Graça et al. (2013) reported a lower *E. coli* H157:O7 reduction in fresh-cut apples after UV-C treatment compared to whole apples spot-inoculated (Yaun et al., 2004) and similarly treated which could be owed to a higher microbial attachment in those fresh-cut apples compared to whole apples related to different product surface characteristics. However, surface inoculation by immersion represents better simulate possible natural pathogen contamination during washing step of fresh-cut products.

Generally, the use of individual strains or a mixture of them for the fresh-cut produce inoculation did not have a great influence on the effectiveness of UV-C treatment (Graça et al., 2013; Schenk et al., 2008). It could be hypothesized that the effectiveness of a UV-C treatment may depend on the inoculum concentration since Beuchat et al. (2004) concluded that *L. monocytogenes* reductions in inoculated lettuce were generally higher as the inoculum level increased using different chemical treatments. However, Yaun et al. (2004) stated that no significant differences were found in the ability of UV-C to inactivate a higher population of either *Salmonella* spp or *E. coli* O157:H7 on the surface of green leaf lettuce. Similarly, no clear reduction pattern related to inoculum concentration was found by Chun et al. (2010) and Kim et al. (2009) after UV-C treatments of 1 kJ m^{-2} conducted in similar conditions in fresh-cut salad and fresh-cut clover sprouts inoculated either with *E. coli* H157:O7 or *L. monocytogenes* (Table 1). The produce used for the UV-C treatment also represents an important factor since the product surface may greatly affect the penetration and reflection of UV-C light. Accordingly, lower Salmonella spp. reductions were found in vegetables with cuticles with high reflection properties such as peppers (jalapeño) and tomatoes (Roma), with similar reductions among them, regarding to those of lettuce leafs using similar UV-C doses. Furthermore, unwaxed apples (Red Delicius) showed higher *E. coli* H157:O7 reductions compared to lettuce leafs (Table 1).

Attending to UV-C instrument conditions, two-side UV-C treatment achieved greater population decreases than one-side treatment in *E. coli* O157:H7, *S. Typhimurium* and *L. monocytogenes* inoculated in fresh-cut lettuce (Kim et al., 2013). This could be related to the inability of UV-C irradiation to go through thin leafy vegetable tissues such as lettuces. The distance between sample and lamps also plays a great role in the UV-C effectiveness. About 1 log CFU g^{-1} greater reduction was yielded at 10-cm compared to 50 cm in fresh-cut lettuce inoculated either with *E. coli* O157:H7, *S. Typhimurium* or *L. monocytogenes* (Kim et al., 2013). However, in the latter work it was not specified if the lamp intensity was also adjusted to be the same for both distances in order to discard the effect of a greater intensity at closer distances. Accordingly, Graça et al. (2013), when inoculating fresh-cut apples, found 0.78 and 0.3 log CFU g^{-1} higher reductions at 15 cm for *E. coli* O157:H7 and *S.* Enteritidis, respectively, than the same treatment conducted by Kim et al. (2009) at 18 cm of distance, although these authors were inoculating the pathogen species in fresh-cut clover sprouts (Table 1). The UV dose is directly proportional to the product of UV intensity and exposure time according to the equation $D = I \cdot t$ where D = applied dose, I = applied intensity and t = exposure time (Environmental Protection Agency, 2006). The UV intensity and exposure time, and consequently the UV-C dose, are fundamental properties of UV light that greatly determine the UV sanitation. Many works found that increasing the UV intensity and/or the exposure time was correlated with increased pathogen reductions in fresh-cut produce. Accordingly, similar levels of inactivation were observed with a given UV dose (8.16 kJ m^{-2}) of 13.6 W m^{-2} for 10 min, and 27.2 W m^{-2} for 5 min in fresh-cut lettuce inoculated with *E. coli* H157:O7, *Salmonella* or *L. monocytogenes* (Kim et al., 2013). Furthermore, increasing the UV-C dose from 1 up to 8 kJ m^{-2} in fresh-cut salad inoculated with *E. coli* H157:O7 led to a proportional inoculum inactivation (Chun et al., 2010). Increasing the UV-C dose from 0.45 kJ m^{-2} to 2.70 also induced a 0.28 log CFU g^{-1} higher reduction of *E. coli* H157:O7 inoculated in fresh-cut mushrooms (Guan et al., 2013). However, other authors did not find a proportional greater reduction of inoculated pathogens in different fresh-cut produce as the UV-C was increased (Escalona et al., 2010; Graça et al., 2013; Yaun et al., 2004).

A valuable review of the effects of UV-C on those pathogens related to outbreaks and consequently legislated (Regulation EC 1441/2007, 2007) for fresh-cut products is presented in Table 1. Based on this review, the best inoculation conditions of the produce and UV-C treatment parameters can be easily selected in further research according to each particular situation. Accordingly, the best experiment conditions were selected in the present work and the inactivation of *E. coli*, *S.* Enteritidis and *L. monocytogenes* by different UV-C doses in inoculated fresh-cut kailan-hybrid broccoli was modelled. The growth of these inoculated pathogens during shelf life at 5, 10 and 15 °C was also studied.

2. Materials and methods

2.1. Plant material and minimal processing

Kailan-hybrid broccoli (*B. oleracea* Italica Group × Alboglabra Group, cv. Bimi®) sized 15–18 cm long was hand-harvested in