Food Control 92 (2018) 312–318

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Inhibition of *Salmonella* Typhimurium growth in coconut (*Cocos nucifera* L.) water by hurdle technology

S. Beristaín-Bauza [a], A. Martínez-Niño [a], A.P. Ramírez-González [a], R. Ávila-Sosa [a], H. Ruíz-Espinosa [b], I.I. Ruiz-López [b], C.E. Ochoa-Velasco [a, *]

[a] *Departamento de Bioquímica-Alimentos, Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, 14 Sur y Av. San Claudio, Ciudad Universitaria, Col. San Manuel, 72420, Puebla, Mexico*

[b] *Ingeniería en Alimentos, Facultad de Ingeniería Química, Benemérita Universidad Autónoma de Puebla, 14 Sur y Av. San Claudio, Ciudad Universitaria, Col. San Manuel, 72420, Puebla, Mexico*

ARTICLE INFO

Article history:
Available online 8 May 2018

Keywords:
Coconut water
UV-C light
S. Typhimurium
Hurdle technology
Shelf life
Beta model

ABSTRACT

The aim of this study was to evaluate a hurdle technology approach (ultraviolet-C light treatment, vanillin or cinnamaldehyde and storage temperature) for preventing the growth of *Salmonella* Typhimurium inoculated into coconut water. Inoculated coconut water was treated with UV-C light at different times (3.5, 7 and 10.5 min); immediately, selected natural antimicrobials (vanillin and cinnamaldehyde) were added at fixed concentrations and stored under refrigeration (5 °C) and room (22 °C) temperatures for 30 days. Beta model was used for describing either growth or death kinetics of *S.* Typhimurium. By themselves, antimicrobials significantly decreased *S.* Typhimurium counts achieving a maximum reduction of 1.4 ± 0.3 log CFU mL^{-1} with cinnamaldehyde ($100\ \mu\text{g mL}^{-1}$). Natural antimicrobials and low temperature significantly affected ($p < 0.05$) *S.* Typhimurium growth during storage. A 7-min UV-C light exposure allowed the inhibition of *S.* Typhimurium growth in coconut water containing natural antimicrobials over a 30-day storage at 5 °C. Moreover, a 10.5-min UV-C light treatment per se prevented *S.* Typhimurium growth in coconut water, regardless of any other hurdles applied. Beta model satisfactorily fits the experimental data. Results might be used for developing a low-cost preservation method for coconut water.

1. Introduction

Coconut water is a sweet clear liquid obtained from the endosperm of coconut fruit; it is a refreshing drink consumed in some tropical countries (Prades, Dornier, Diop, & Pain, 2012a). As the consumption of natural and healthy beverages has been on the rise in recent years, coconut water could potentially be commercialized worldwide (Prades, Dornier, Diop, & Pain, 2012b). Different compounds in coconut water, such as soluble sugars, minerals, vitamins, phytohormones and amino acids, have exhibited antioxidant, cardio protective, anticancer, antifungal, antiviral, antiparasitic and hypoglycemic activities which could positively impact human health (DebMandal & Mandal, 2011; Kalman, Feldman, Krieger, & Bloomer, 2012; Preetha, Devi, & Rajamohan, 2012; Yong, Ge, Ng, & Tan, 2009). Nevertheless, some preservation issues must be solved first, as coconut water is a suitable medium for microbial growth, specifically enterobacteria such as *Escherichia* and *Salmonella* (Awua, Doe, & Agyare, 2012).

Thermal treatment has been widely applied for reducing the microbial load in foodstuffs and its deleterious activity during storage. However, heat may affect product quality (González-Cebrino, García-Parra, Contador, Tabla, & Ramírez, 2012). Therefore, non-thermal treatments have been explored as alternatives to ensure food safety without modifying desirable physicochemical, nutritional and sensory properties. In this aspect, short-wave ultraviolet-C (UV-C) light, a common treatment used to disinfect water and surfaces, has been recently applied to pasteurize fluid foods such as liquid egg (Unluturk, Atilgan, Baysal, & Tari, 2008) and juices (Carrillo, Ferrario, & Guerrero, 2017). The effect of UV-C light on microorganisms is at DNA level; UV-C light photons may penetrate the cell membranes and produce pyrimidine dimers,

---

* Corresponding author. Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla, Mexico.

E-mail addresses: carlosenriqueov@hotmail.com, carlos.ochoav@correo.buap.mx (C.E. Ochoa-Velasco).

https://doi.org/10.1016/j.foodcont.2018.05.010

which block the DNA replication and transcription, resulting in cell death (Hernández-Carranza et al., 2016). Nevertheless, some microorganisms can repair DNA damage (photoreactivation and/or dark repair) if the UV-C light treatment applied is insufficient to completely inactivate them (Ochoa-Velasco et al., 2018). Exploring its use in coconut water processing, Gabriel (2015) pointed out that *Salmonella enterica* is an appropriate reference microorganism for assessing the effectiveness of UV-C light as a preservation factor.

Microbial predictive modeling was developed to describe the effect of preservation factors such as temperature, pH, antimicrobial agents or water activity on growth or inactivation of microorganisms (Alzamora et al., 2010). Several models may be useful to obtain kinetic parameters related to microbial inactivation (maximum microbial death, death rate, decimal reduction time, etc.) or growth (latency time, maximum microbial growth, duplication time, among others), which in turn can be used to evaluate treatment efficiency or to predict shelf life of food products (Ochoa-Velasco et al., 2018). Although several empirical models have been reported in the literature, some of them need to be modified to depict growth or death kinetics. Originally proposed by Yin, Goudriaan, Lantinga, Vos, and Spiertz (2003) to describe growth parameters of grains and seeds, the Beta model has been shown to successfully fit microbial growth-death kinetics (Buzrul, 2009; Ochoa-Velasco et al., 2018).

In addition to their well-characterized activity against different spoilage and pathogenic microorganisms, natural antimicrobials are currently favored over synthetic ones because of consumer concerns over possible health effects of the latter (Espina et al., 2011; Gutierrez, Barry-Ryan, & Bourke, 2008; Negi, 2012). However, high concentrations of natural antimicrobials are required to inhibit microbial growth, which may impart undesirable flavors to food products (Samant et al., 2015). Among natural antimicrobials, aldehydes as vanillin and cinnamaldehyde are widely used because of their effectiveness and contribution to sensory quality. Vanillin, the major flavor compound of vanilla beans, is used in a broad variety of products including baked goods, ice creams, beverages, among others (Char, Guerrero, & Alzamora, 2009). On the other hand, cinnamaldehyde is an aromatic α-β-unsaturated aldehyde, and the major component of bark cinnamon essential oil (Baskaran, Amalaradjou, Hoagland, & Venkitarayanan, 2010). Vanillin and cinnamaldehyde are generally recognized as safe by the FDA and have been evaluated alone or in combination with other preservation factors against microbial growth in selected liquid foods, e.g. vanillin was satisfactory evaluated against *E. coli* 0157:H7 and *L. innocua* in apple and orange juices, respectively (Char et al., 2009; Moon, Delaquis, Toivonen, & Stanich, 2006), while cinnamaldehyde proved to be effective against *E. coli* O157:H7 (Baskaran et al., 2010) and *Salmonella enterica* inoculated in apple juice (Friedman, Henika, Levin, & Mandrell, 2004).

Nowadays health-conscious consumers are looking for nutritious, fresh-like, minimally processed foods with high sensory quality. Consequently, a hurdle technology approach, where product shelf life is extended without losing its nutritional and sensory quality, has been explored (Carrillo et al., 2017; Cassani, Tomadoni, Ponce, Agüero, & Moreira, 2017; Chen, Luo, Luo, & Zhu, 2017; Espina et al., 2011), frequently using natural antimicrobials as one of the hurdles. In this sense, Cassani et al. (2017) explored the combined use of ultrasound and vanillin as a preservation alternative for strawberry juice; previously, Gabriel and Estilo (2015) evaluated the effect of malic acid and/or nisin with mild thermal treatment (55 °C) against *E. coli* O157:H7 inoculated in coconut water. Thus, the combination of natural antimicrobials in appropriate concentrations with other mild treatments may constitute a suitable way to reduce microbial load while enhancing shelf life of various foodstuffs. As far as we know, the evaluation of combined UV-C light treatments and natural antimicrobials for preserving coconut water have not been reported. Therefore, the aim of this study was to evaluate the use of a hurdle technology approach (Ultraviolet-C light treatment, vanillin or cinnamaldehyde and storage temperature) on the growth of *S.* Typhimurium inoculated in coconut water.

2. Materials and methods

2.1. Coconut water

Fresh young coconut fruits (*Cocos nucifera* L.) were acquired from a local supermarket in Puebla City, Puebla, Mexico. Coconut fruits were washed with distilled sterile water and neutral detergent, surface-disinfected with ethanol (70%/1 min) and air-dried. The disinfected fruits were then aseptically perforated with a knife to obtain their water (500–600 mL water per coconut fruit). Total soluble solids (TSS) and pH of coconut water were determined with a hand refractometer (Atago, Tokyo, Japan) and a pH-meter (Orion 420 A, Massachusetts, USA), respectively. The UV-C absorbance (254 nm) of coconut water was evaluated using a Jenway spectrophotometer (model 6405, Felsted, UK).

2.2. Salmonella Typhimurium growth

Salmonella enterica subsp. enterica serovar Typhimurium (ATCC 14028) was obtained from the microbial culture collection at Benemérita Universidad Autónoma de Puebla (Puebla City, Puebla, Mexico). The microorganism was previously grown in nutritive broth (BD Bioxon, Mexico City, Mexico) during 24 h at 37 ± 2 °C (stationary phase). Then, 1 mL of this culture was added to 100 mL of coconut water and incubated for 24 h (37 ± 2 °C).

2.3. Ultraviolet-C light equipment

The annular UV-C light equipment used in this study is composed of a feeding tank, a peristaltic pump (Master Flex, Vernon, Illinois, USA), and UV-C light lamps (TodoAgua, San Luis Potosi, Mexico) each with a 0.4-L capacity (Fig. 1). The UV-C lamp (254 nm; 12W UV-C output) was inserted into a 29.3-cm length, 2.3-cm inner diameter quartz sleeve with an outer stainless-steel sleeve (29.5 cm × 4.8 cm). UV-C light dose ($\mathrm{J\ cm^{-2}}$) was calculated according to Equation (1) (Keyser, Műller, Cilliers, Nel, & Gouws, 2008).

$$F = I \times t \tag{1}$$

where F is the UV-C irradiation dose ($\mathrm{J\ cm^{-2}}$), I is the lamp intensity ($\mathrm{W\ cm^{-2}}$), and t is the residence time (s).

2.4. Antimicrobials

Trans-cinnamaldehyde and vanillin were obtained from Sigma-Aldrich (Toluca, Mexico). Aqueous solutions of both were prepared at a concentration of 1000 $\mu\mathrm{g\ mL^{-1}}$ using tween 20 (Sigma-Aldrich, Toluca, Mexico) as emulsifier (1% v/v). Solutions were sterilized through a 0.2-μm Acrodisc® syringe filter (Sigma-Aldrich, Toluca, Mexico) and refrigerated (5 °C) in an amber glass tube until used. Preliminary microbial and sensory tests were conducted to determine the vanillin and cinnamaldehyde levels to be used as hurdles. In both tests, fresh coconut water added with either vanillin or cinnamaldehyde (25, 50 or 100 $\mu\mathrm{g\ mL^{-1}}$) was used. For microbial tests, coconut water was inoculated with *S.* Typhimurium and its microbial load was analyzed after a 24 h-storage at 37 ± 2 °C. For sensory evaluation, 15-mL samples of coconut water were

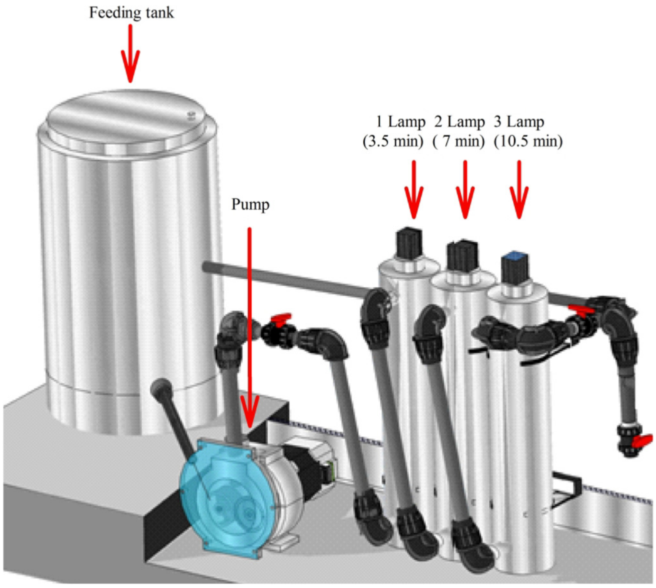

Fig. 1. Ultraviolet-C light processing equipment for liquids.

presented to 100 untrained judges to evaluate its overall acceptability using a 9-point hedonic scale where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely.

2.5. Hurdle technologies

Twenty-mL samples of inoculated coconut water were added to fresh coconut water (2 L) to reach a microbial load of 1.0×10^6 colony forming units per mL (CFU mL^{-1}). Inoculated coconut water was then treated with UV-C light for 10.5 min at a constant flow rate of 1.9 mL s^{-1}. Samples were taken at 0, 3.5, 7, and 10.5 min, times equivalent to doses of 0, 9.24, 18.48, and 27.72 J cm^{-2}, respectively. Then, 100 μg mL^{-1} of vanillin or cinnamaldehyde were added to coconut water and stored in glass bottles either under refrigeration (5 ± 1 °C) or at room (22 ± 2 °C) temperatures. *S.* Typhimurium count was performed at 0, 1, 2, 4, 8, 16, and 30 days of storage. Coconut water samples without UV-C light treatment or without added antimicrobials were used as controls. Treatments were performed in triplicate and analysis in duplicate.

2.6. Salmonella Typhimurium counts

Serial dilutions of coconut water in peptone water were made until the appropriate concentration (30–300 CFU mL^{-1}) was attained. Dilutions were plated on Salmonella-Shigella agar (BD Bioxon, Mexico City, Mexico), incubated at 35 ± 2 °C and enumerated after 24 h. Results were expressed as CFU mL^{-1}.

2.7. Microbial kinetics modeling

The effect of the above described hurdle technology approach on *S.* Typhimurium growth during coconut water storage was evaluated using the Beta function (Eq. (2)).

$$log\,\frac{N}{N_0} = A\left(1 + \frac{t_A - t}{t_A - t_m}\right)\left(\frac{t}{t_A}\right)^{\frac{t_A}{t_A - t_m}} \tag{2}$$

where N_0 is the initial microbial load (CFU mL^{-1}) and N is the microbial load (CFU mL^{-1}) at any given storage time (t), A is the maximum microbial growth achieved at time t_A; t_m is the time for maximum growth rate (μ_{max}) (Eq. (3)). The lag time τ ($t_m > 0$) for Beta model was calculated as proposed by Ochoa-Velasco et al. (2018) with (Eq. (4)).

$$\mu_{max} = A\left[\frac{2t_A - t_m}{t_A(t_A - t_m)}\right]\left(\frac{t_m}{t_A}\right)^{\frac{t_m}{t_A - t_m}} \tag{3}$$

$$\tau = \frac{t_m^2}{2t_A - t_m} \tag{4}$$

2.8. Statistical analysis

Estimation of model parameters (nonlinear regression based on ordinary least squares) and statistical analyses were performed with Matlab software using the Statistics Toolbox 7.3 (Matlab R2010a, MathWorks Inc., Natick, MA, USA).

3. Results and discussion

3.1. Effect of antimicrobial concentration on S. Typhimurium growth and overall acceptance of coconut water

Fig. 2 shows the effect of vanillin and cinnamaldehyde against *S.* Typhimurium inoculated in coconut water. *S.* Typhimurium load was significantly reduced ($p < 0.05$) at the highest concentration (100 μg mL^{-1}) of both vanillin (0.7 ± 0.2 log cycles) and cinnamaldehyde (1.4 ± 0.3 log cycles). Although the mechanism of natural antimicrobials action is not known in detail, reports indicate that vanillin and cinnamaldehyde may affect the cytoplasmic membrane permeability (Rupasinghe, Boulter-Bitzer, Ahn, & Odumeru, 2006). Moreover, the carbonyl group of cinnamaldehyde binds to proteins, affecting the amino acid decarboxylase activity. Both mechanisms conduct to cell death (Wendakoon & Sakaguchi, 1995). In the current study, cinnamaldehyde-induced microbial inactivation was similar to that reported by Zhou et al. (2007) where a 1.1-log cycle reduction of *S.* Typhimurium CGMCC 1.1174 in Mueller Hinton broth was attained by using cinnamaldehyde at the same concentration (100 μg mL^{-1}). In comparison, several reports indicate that vanillin exhibits a lower antimicrobial activity in food systems; consequently, higher concentrations are needed to reduce the microbial load (Rupasinghe et al., 2006).

One of the main disadvantages of using natural antimicrobials in

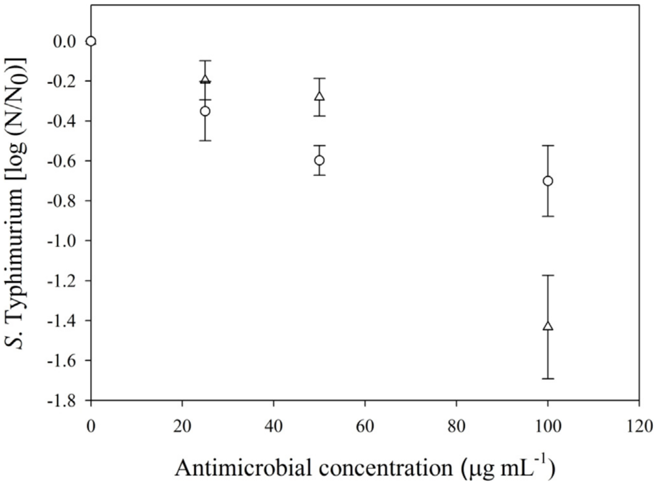

Fig. 2. Effect of vanillin (○) and cinnamaldehyde (Δ) against *Salmonella* Typhymurium inoculated in coconut water after 24 h of storage. Bars indicate standard deviation.

food formulations is the undesirable sensory characteristics they impart when used in high concentrations. In order to evaluate the feasibility of using vanillin and cinnamaldehyde in coconut water, a sensory evaluation was performed. Table 1 shows the overall acceptability of fresh and antimicrobial-containing coconut water. Coconut water acceptability fell between like slightly and like moderately on the hedonic scale. A similar overall acceptability (6.3 ± 1.4) was reported by Adubofuor, Amoah, and Osei-Bonsu (2016) for untreated green coconut water, while heat treatment (90 °C, 5 min) did not significantly reduce its acceptability. On the other hand, the addition of natural antimicrobials at the tested levels did not significantly affect ($p < 0.05$) product acceptability. Moreover, the acceptability category with the highest frequency response was "like moderately" (>20%), probably due to the pleasant taste provided by vanillin and cinnamaldehyde. In a similar study, Espina et al. (2012) pointed out that the addition of essential lemon oil (75 $\mu L\ L^{-1}$) did not decrease the acceptability of apple juice when compared with an untreated counterpart. Sensory results obtained indicate that vanillin and cinnamaldehyde could be used at a concentration as high as 100 $\mu g\ mL^{-1}$ without compromising coconut water acceptability. Therefore, this concentration was selected to evaluate the effect of vanillin and cinnamaldehyde as hurdles against *S.* Typhimurium after UV treatment and during the storage of coconut water.

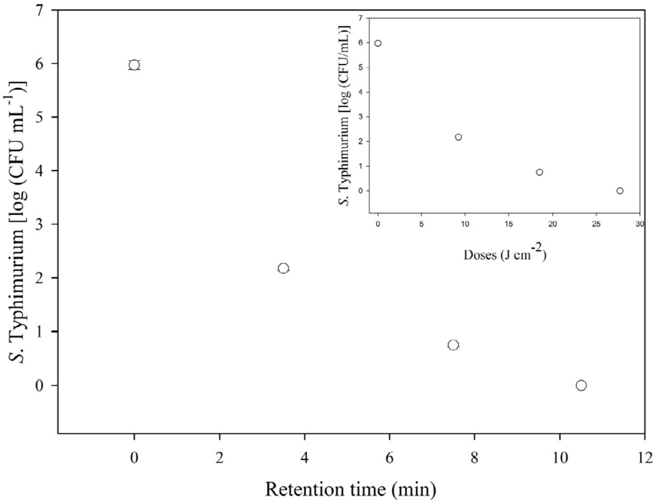

Fig. 3. Effect of UV-C light treatment against *Salmonella* Typhymurium inoculated in coconut water. Bars indicate standard deviation.

3.2. Ultraviolet-C light effect on S. Typhimurium inoculated in coconut water

Fig. 3 shows the effect of UV-C light treatment on *S.* Typhimurium inoculated in coconut water. As observed, by increasing UV-C light treatment time, a higher logarithmic reduction is achieved. After 3.5-min and 7-min UV-C light processes, a microbial reduction of 3.8 ± 0.1 and 5.2 ± 0.1 log cycles was obtained, respectively. On the other hand, a 10.5 min UV-C light exposure produced a complete inhibition (>6 log units) of *S.* Typhimurium in coconut water. As it has been previously shown, the effects of UV-C light treatment on microbial inactivation depend on several intrinsic and extrinsic factors including physicochemical and optical properties of liquid foods, microbial-related features, UV-C light dose and equipment characteristics, among others (Gayán, Serrano, Raso, Álvarez, & Condón, 2012; Koutchma, Forney, & Moraru, 2009). For example, a considerable higher microbial inactivation rate of *Salmonella enterica* was reported by Gabriel and Colambo (2016) for UV-C treated coconut liquid endosperm; such disparities in UV-C induced lethality are somehow justified due to the physicochemical characteristics (pH 5.3 ± 0.1, TSS 4.4 ± 0.3%, and UV-C light absorbance 1.25 ± 0.01) of coconut water used in this study and the UV-C light equipment design and operation (a stirred tank vessel versus a continuous flow system).

3.3. Effect of hurdle technology against S. Typhimurium growth in stored coconut water

The effect of a hurdle technology approach (UV-C light, natural antimicrobials and storage temperature) on growth of *S.* Typhimurium inoculated in coconut water is shown in Fig. 4. Untreated inoculated coconut water exhibited a maximum growth of 6.0 log cycles (CFU mL^{-1}); a similar result was reported by Gabriel (2015) in coconut water inoculated with *S. enterica*. Meanwhile, coconut water inoculated with *E. coli* and *K. pneumoniae* presented a maximum microbial growth of 2.27 and 2.83 × 10^8 CFU/mL, respectively (Awua et al., 2012). Thus, coconut water can be considered a suitable medium for microbial growth. Besides, *S.* Typhimurium growth in coconut water during a 30-day storage period was followed; although microbial population was expected to increase over time, after only 8 days, microbial load started decreasing, which could be attributed to nutrient exhaustion in the food matrix and cells entering death phase (Maier, 2000). On the other hand, all hurdles used for coconut water preservation significantly affected ($p < 0.05$) *S.* Typhimurium growth during storage. Both storage temperature and natural antimicrobials reduced *S.* Typhimurium growth in coconut water. In this regard, cinnamaldehyde showed the highest antimicrobial activity against *S.* Typhimurium. Although Gabriel and Pineda (2014) evaluated the combined effect of mild heat treatment plus vanillin as hurdles to inactivate *E. coli* O157:H7 in coconut water, the use of vanillin or cinnamaldehyde as preservation factors to increase coconut water shelf life has not been reported. Manu et al. (2017) reported that *S. enterica* was completely inactivated by cinnamaldehyde and low temperature storage in carrot (2.0 $\mu L\ mL^{-1}$ of cinnamaldehyde) and berry (1.5 $\mu L\ mL^{-1}$ of cinnamaldehyde) juices after being held at 4 °C for a few hours. In a similar way, Cassani et al. (2017) indicated that vanillin significantly reduced the native microbial population (4–6 log cycles) of strawberry juice during storage.

As mentioned before, UV-C light treatment significantly

Table 1
Overall acceptability of coconut water added with vanillin or cinnamaldehyde.

Antimicrobial	Concentration ($\mu g\ mL^{-1}$)	Overall	Acceptability mode acceptability[a]
None	–	6.2 (5.8/6.6)	8-Like much (23%)
Vanillin	25	6.0 (5.5/6.5)	8-Like much (21%)
Vanillin	50	6.0 (5.5/6.4)	8-Like much (22%)
Vanillin	100	5.4 (4.9/5.8)	7-Like moderately (22%)
Cinnamaldehyde	25	6.5 (6.1/6.7)	7-Like moderately (26%)
Cinnamaldehyde	50	6.4 (6.0/6.8)	8-Like much (26%)
Cinnamaldehyde	100	6.1 (5.6/6.5)	8-Like much (20%)

[a] Average (95% Confidence interval).

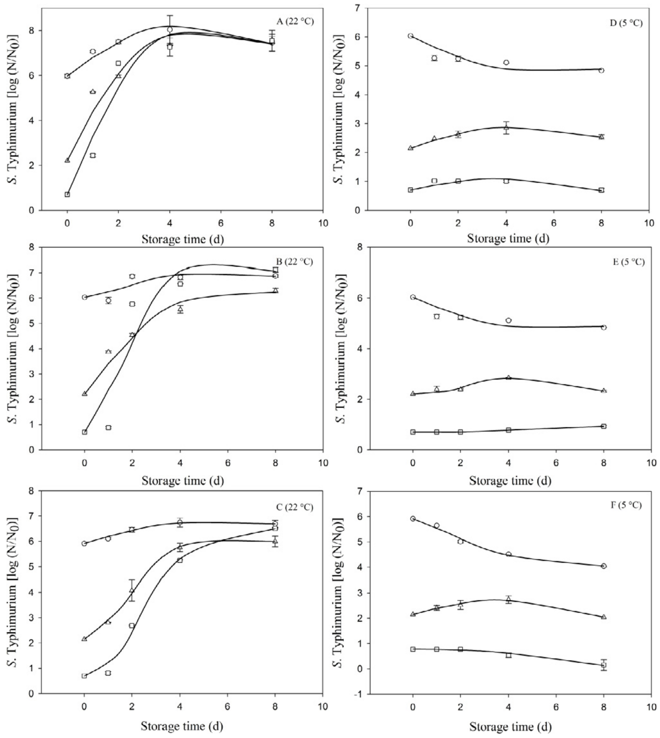

Fig. 4. Effect of hurdle technology on *Salmonella* Typhimurium growth in coconut water during storage. Antimicrobials: Control (Figure A and D), vanillin (Figure B and E) and cinnamaldehyde (Figure C and F). Treatments: without UV-C light (○), 3.5 min (Δ) and 7.0 min (□) of UV-C light. Black lines are the fitting data to Beta model. Bars indicate standard deviation.

($p < 0.05$) reduced *S.* Typhimurium counts in coconut water. However, after UV-C light treatment, *S.* Typhimurium growth behavior changed depending on the obstacles used. In coconut water stored at 22 °C (Fig. 4A, B, and C), *S.* Typhimurium load increased during storage while a lower microbial growth was observed in coconut water added with natural antimicrobials. It is interesting to note that untreated and UV-C light-treated (3.5 and 7 min) coconut water showed a similar microbial count after 8 days of storage, regardless of the use of antimicrobials. In a recent study, Ochoa-Velasco et al. (2018) reported that if *S.* Typhimurium cells (<10 CFU mL^{-1}) remain viable in coconut water after UV-C light treatment, they can reach a high microbial load in few days due to photoreactivation and/or dark repair mechanisms (Nebot Sanz, Salcedo Dávila, Andrade Balao, & Quiroga Alonso, 2007). Thus, keeping coconut water at low temperatures during storage is paramount to avoid or delay *S.* Typhimurium growth after the application of UV-C light. Fig. 4D, E and F show the *S.* Typhimurium growth in coconut water stored at 5 °C with and without adding vanillin or cinnamaldehyde, respectively. Regardless of the addition of natural antimicrobials, a similar microbial growth was observed in 3.5 min- UV-C treated coconut water after 8 days of storage. All systems presented an increase of 0.59–0.61 log cycles of *S.* Typhimurium after 4 days of storage with a slight reduction in microbial counts thereafter. Coconut water treated with UV-C light for 7 min did not present any significant increase ($p < 0.05$) in *S.* Typhimurium growth after 8 days; meanwhile, cinnamaldehyde addition reduced the microbial load to less than 10 CFU mL^{-1}. No microbial growth was detected during the storage of coconut water treated at the highest UV-C light exposure, independently of the antimicrobials or the storage temperature used. Similar results were reported by Antonelli, Mezzanotte, and Nurizzo (2008). They informed that at low UV-C light doses, a reactivation process of enterobacteriaceae (*E. coli*, total and fecal coliforms) may occur; however, when UV-C light dose is increased, the damage caused is so severe that cells are unable to repair it. On the other hand, although the microbial growth during storage was not analyzed, Gautam et al. (2017) pointed out that coconut water treated in a continuous flow coiled UV-C reactor maintained its quality characteristics for as long as 28 days.

The kinetic parameters of Beta model provided valuable information on the effect of the hurdle technology approach on *S.* Typhimurium growth inoculated in coconut water (Table 2). Beta model could reproduce experimental data ($R^2 > 0.77$) and all estimated parameters were significant ($p < 0.05$) indicating that the proposed equation was not overfitted, regardless of the type of kinetic data. Untreated coconut water stored at 22 °C exhibited a lower maximum microbial load (A) than in UV-C light treated coconut water at all sampling times; this might indicate that, independently of the UV-C light treatment dosage applied to coconut water, if *S.* Typhimurium was not completely inactivated, it could repair the UV-C induced DNA damage, reaching a higher a microbial load than its untreated counterpart in a few days. Negative A values were observed in all untreated coconut water stored at 5 °C, which in turn indicate a significant microbial reduction during storage ($p < 0.05$); although *S.* Typhimurium growth was observed in UV-C light treated coconut water (positive A values). In this aspect, Lasagabaster and Martínez de Marañón (2017) reported a high microbial reduction (>6 log cycles) of *Listeria* genus bacteria by pulsed light, although a photoreactivation process associated to the photolyase enzyme was presumed. In general, the time to reach the maximum microbial growth (t_A) was similar in all treatments ranging from 3.95 to 7.01 days; however, *S.* Typhimurium in 7-min UV-C light-treated coconut water added with cinnamaldehyde and stored at 5 °C showed the shortest t_A (0.01 days) and the minimum value of maximal microbial load (3.3×10^{-7}), indicating that it did not grow during storage. It is important to point out that t_A values of untreated coconut water stored at 5 °C indicate the time to reach the maximum reduction of *S.* Typhimurium. Although, the time (t_m) for maximum microbial growth rate did not show any clear trend, 7-min UV-C light-treated coconut water with added vanillin stored at 5 °C presented the highest t_m (5.81 days) and consequently the longest lag phase (4.10 days).

Negative values for the maximum microbial growth rate (μ_{max}) were observed in untreated coconut water stored at low temperature (regardless of the use of antimicrobials) indicating a mild inactivation of the microbial population during storage. Moreover, a direct relationship between UV-C light treatment time and *S.* Typhimurium growth rate was observed in coconut water stored at 22 °C. This may be associated to the stress caused by the UV-C light, which might stimulate DNA repair synthesis mostly by photoreactivation, excision repair (dark repair), mismatch repair and SOS (save our soul), among other mechanisms (Rastogi, Richa, Kumar, Tyagi, & Sinha, 2010). Janion (2008) informed that *S.* Typhimurium has a SOS response; at normal conditions, these SOS genes (LexA and RecA proteins) are expressed at basal level, but under some mutagenesis conditions, SOS genes response increases the production of proteins relevant to DNA protection and repair. A comparable result was obtained by Nebot Sanz et al. (2007) where the kinetic rate constant augmented by increasing the UV-C light dose against enterobacteriaceae (total and fecal coliforms and *Streptococcus faecalis*).

4. Conclusions

The use of vanillin and cinnamaldehyde at the tested level reduced *S.* Typhimurium load on their own without affecting the overall acceptability of coconut water. UV-C light treatment

Table 2
Beta model parameters of *S.* Typhimurium growth in coconut water treated with hurdle technology.

RT[a] (Dose[b])	Antimicrobial (100 μg mL^{-1})	Temperature (°C)	A[c]	t_A[c] (d)	t_m[c] (d)	τ[d] (d)	μ_{max}[c]	R^2 (d^{-1})
0 (0)	None[e]	5	−1.10 (−1.19/-1.00)	6.54 (4.38/8.69)	–	–	−0.34 (−0.43/-0.26)	0.847
0 (0)	Vanillin[e]	5	−1.29 (−1.36/-1.22)	6.05 (5.62/6.49)	–	–	−0.43 (−0.48/-0.37)	0.813
0 (0)	Cinnamaldehyde	5	−1.90 (−1.93/-1.86)	7.25 (7.17/7.33)	0.99 (0.71/1.28)	0.07	−0.41 (−0.42/-0.40)	0.988
0 (0)	None[e]	22	2.32 (1.75/2.89)	5.10 (4.23/5.97)	–	–	0.93 (0.55/1.31)	0.944
0 (0)	Vanillin	22	1.04 (1.00/1.09)	5.89 (5.33/6.44)	1.30 (0.50/2.09)	0.17	0.27 (0.22/0.32)	0.745
0 (0)	Cinnamaldehyde[e]	22	0.90 (0.76/1.05)	5.84 (5.36/6.42)	–	–	0.31 (0.29/0.33)	0.957
3.5 (9.2)	None	5	0.67 (0.66/0.69)	5.11 (4.76/5.48)	1.81 (1.12/2.50)	0.40	0.19 (0.18/0.20)	0.990
3.5 (9.2)	Vanillin	5	0.76 (0.59/0.92)	5.38 (4.75/6.01)	2.53 (1.07/4.00)	0.85	0.21 (0.17/0.25)	0.920
3.5 (9.2)	Cinnamaldehyde[e]	5	0.56 (0.37/0.75)	3.83 (3.77/3.89)	–	–	0.29 (0.20/0.39)	0.933
3.5 (9.2)	None[e]	22	5.69 (5.59/5.80)	4.63 (4.53/4.74)	–	–	2.46 (2.45/2.47)	0.957
3.5 (9.2)	Vanillin[e]	22	4.27 (4.26/4.27)	6.50 (6.21/6.78)	–	–	1.31 (1.26/1.37)	0.976
3.5 (9.2)	Cinnamaldehyde	22	4.51 (4.50/4.52)	6.21 (5.79/6.65)	1.74 (1.15/2.33)	0.29	1.06 (0.97/1.15)	0.995
7 (18.5)	None[e]	5	0.38 (0.38/0.39)	3.95 (3.76/4.14)	–	–	0.19 (0.18/0.21)	0.773
7 (18.5)	Vanillin	5	0.59 (0.54/0.65)	7.01 (6.98/7.05)	5.81 (5.78/5.84)	4.10	0.23 (0.20/0.26)	0.999
7 (18.5)	Cinnamaldehyde[e]	5	[3.3 (1.8/4.7)] × 10^{-7}	[5.9 (3.2/8.6)] × 10^{-3}	–	–	[1.1 (1.1/1.1)] × 10^{-4}	0.974
7 (18.5)	None[e]	22	7.96 (7.91/8.01)	5.80 (5.36/6.22)	–	–	2.75 (2.53/2.97)	0.930
7 (18.5)	Vanillin	22	7.46 (7.42/7.50)	6.04 (6.00/6.09)	1.07 (1.00/1.15)	0.10	1.88 (1.87/1.90)	0.879
7 (18.5)	Cinnamaldehyde	22	6.70 (6.59/6.80)	6.59 (6.55/6.63)	3.00 (2.90/3.09)	0.88	1.49 (1.45/1.54)	0.991

[a] Residence time (min).
[b] J cm^{-2}.
[c] Average (95% Confidence interval).
[d] Estimated with Eq. (4).
[e] Parameter t_m and τ were set to zero.

significantly reduced ($p < 0.05$) *S.* Typhimurium load in coconut water. Natural antimicrobials and low temperature storage significantly affected *S.* Typhimurium growth in the tested product. Moreover, by increasing UV-C light treatment time, higher maximum microbial growth and growth rate of *S.* Typhimurium in coconut water were observed. 7-min UV-C light-treated coconut water stored at 5 °C and added with vanillin or cinnamaldehyde showed a 4-day lag phase or did not present *S.* Typhimurium growth for 30 days, respectively; also, 10.5-min UV-C light-treated coconut water did not present any *S.* Typhimurium growth during the same storage time, regardless the use of natural antimicrobial or low temperature storage. This study clearly indicates that a preservation strategy based on properly selected and combined hurdles may be useful to enhance coconut water shelf life.

Acknowledgments

The author Dr. Silvia del Carmen Beristaín Bauza would like to thank the Secretaría de Educación Pública for her fellowship grant (511-6/17-10082) and the Benemérita Universidad Autónoma de Puebla for the support provided during her posdoctoral studies.

References

Adubofuor, J., Amoah, I., & Osei-Bonsu, I. (2016). Sensory and physicochemical properties of pasteurized coconut water from two varieties of coconut. *Food Science and Quality Management, 54*, 26–32.

Alzamora, S. M., Guerrero, S. N., López-Malo, A., Palou, E., Char, C. D., & Raffellini, S. (2010). Models for microorganism inactivation: Application in food preservation design. In E. Ortega-Rivas (Ed.), *Processing effects on safety and quality of foods* (pp. 87–115). USA: CRC-Press.

Antonelli, M., Mezzanotte, V., & Nurizzo, C. (2008). Wastewater disinfection by UV irradiation: Short and long-term efficiency. *Environmental Engineering Science, 25*, 363–373.

Awua, A. K., Doe, E. D., & Agyare, R. (2012). Potential bacterial health risk posed to consumers of fresh coconut (*Cocos nucifera* L.) water. *Food and Nutrition Sciences, 3*, 1136–1143.

Baskaran, S. A., Amalaradjou, M. A. R., Hoagland, T., & Venkitarayanan, K. (2010). Inactivation of *Escherichia coli* O157:H7 in apple juice and apple cider by trans-cinnamaldehyde. *International Journal of Food Microbiology, 141*, 126–129.

Buzrul, S. (2009). A suitable model of microbial growth. *African Journal of Microbiology Research, 3*, 468–474.

Carrillo, M. G., Ferrario, M., & Guerrero, S. (2017). Study of the inactivation of some microorganisms in turbid carrot-orange juice blend processed by ultraviolet light assisted by mild heat treatment. *Journal of Food Engineering, 212*, 213–225.

Cassani, L., Tomadoni, B., Ponce, A., Agüero, M. V., & Moreira, M. R. (2017). Combined use of ultrasound and vanillin to improve quality parameters and safety of strawberry juice enriched with prebiotics fibers. *Food and Bioprocess Technology, 10*, 1454–1465.

Char, C., Guerrero, S., & Alzamora, S. M. (2009). Survival of *Listeria innocua* in thermally processed orange juice as affected by vanillin addition. *Food Control, 20*, 67–74.

Chen, A. J., Luo, W., Luo, Y. B., & Zhu, B. Z. (2017). Combined treatment of ultraviolet-C and *L. plantarum* on *Salmonella enteritidis* and quality control of fresh-cut apple. *Journal of Food Processing and Preservation, 42*, 1–9.

DebMandal, M., & Mandal, S. (2011). Coconut (*Cocos nucifera* L.: Arecaceae): In health promotion and disease prevention. *Asian Pacific Journal of Tropical Medicine, 4*, 241–247.

Espina, L., Somolinos, M., Lorán, S., Conchello, P., García-Gonzalo, D., & Pagán, R. (2011). Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes. *Food Control, 22*, 896–902.

Espina, L., Somolinos, M., Ouazzou, A. A., Condón, S., García-Gonzalo, D., & Pagán, R. (2012). Inactivation of *Escherichia coli* O157:H7 in fruit juices by combined treatments of citrus fruit essential oils and heat. *International Journal of Food Microbiology, 159*, 9–16.

Friedman, M., Henika, P. R., Levin, C. E., & Mandrell, R. (2004). Antibacterial activities of plant essential oils and their components against *Escherichia coli* O157:H7 and *Salmonella enterica* in apple juice. *Journal of Agricultural and Food Chemistry, 52*, 6042–6048.

Gabriel, A. A. (2015). Previous physicochemical stress exposures influence subsequent resistance of *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* to ultraviolet-C in coconut liquid endosperm beverage. *International Journal of Food Microbiology, 201*, 7–16.

Gabriel, A. A., & Colambo, J. C. R. (2016). Comparative resistances of selected spoilage and pathogenic bacteria in ultraviolet-C-treated, turbulent-flowing young coconut liquid endosperm. *Food Control, 69*, 134–140.

Gabriel, A. A., & Estilo, E. E. (2015). Influences of malic acid and nisin supplementations on the decimal reduction times of *Escherichia coli* O157:H7 in mildly-heated young coconut liquid endosperm. *Food Control, 50*, 645–651.

Gabriel, A. A., & Pineda, J. K. F. (2014). Influences of vanillin and licorice root extract supplementations on the decimal reduction times of *Escherichia coli* O157:H7 in mildly heated young coconut liquid endosperm. *Food Control, 38*, 136–141.

Gautam, D., Umagiliyage, A. L., Dhital, R., Joshi, P., Watsn, D. G., Fisher, D. J., et al. (2017). Nonthermal pasteurization of tender coconut water using a continuous flow coiled UV reactor. *LWT-Food Science and Technology, 83*, 127–131.

Gayán, E., Serrano, M. J., Raso, J., Álvarez, I., & Condón, S. (2012). Inactivation of *Salmonella enterica* by UV-C light alone and in combination with mild temperatures. *Applied and Enviromental Microbiology, 78*, 8353–8361.

González-Cebrino, F., García-Parra, J., Contador, R., Tabla, R., & Ramírez, R. (2012). Effect of high-pressure processing and thermal treatment on quality attributes and nutritional compounds of "Songold" plum purée. *Journal of Food Science, 77*, C866–C873.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*, 91–97.

Hernández-Carranza, P., Ruíz-López, I. I., Pacheco-Aguirre, F. M., Guerrero-Beltrán, J.Á., Ávila-Sosa, R., & Ochoa-Velasco, C. (2016). Ultraviolet-C light effect on physicochemical, bioactive, microbiological, and sensorial characteristics of carrot (*Daucus carota*) beverages. *Food Science and Technology, 22*, 536–546.

Janion, C. (2008). Inducible SOS response system of DNA repair and mutagenesis in *Escherichia coli*. *International Journal of Biological Sciences, 4*, 338–344.

Kalman, S. S., Feldman, S., Krieger, D. R., & Bloomer, R. J. (2012). Comparison of coconut water and a carbohydrate-electrolyte sport drink on measures of hydration and physical performance in exercise-trained men. *Journal of the International Society of Sports Nutrition, 9*, 1–10.

Keyser, M., Műller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science & Emerging Technologies, 9*, 348–354.

Koutchma, T., Forney, L., & Moraru, C. (2009). *Ultraviolet light in food Technology: Principles and applications*. Boca Raton: CRC Press.

Lasagabaster, A., & Martínez de Marañón, I. (2017). Comparative study on the inactivation and photoreactivation response of *Listeria monocytogenes* seafood isolates and a *Listeria innocua* surrogate after pulsed light treatment. *Food and Bioprocess Technology, 10*, 1931–1935.

Maier, R. M. (2000). Bacterial growth. In R. M. Maier, I. L. Pepper, & C. P. Gebra (Eds.), *Environmental microbiology* (pp. 43–59). UK: Academic Press.

Manu, D., Mendonca, A. F., Daraba, A., Dickson, J., Sebranek, J., Shaw, A., et al. (2017). Antimicrobial efficacy of cinnamaldehyde against *Escherichia coli* O157:H7 and *Salmonella enterica* in carrot juice and mixed berry juice held at 4°C and 12°C. *Foodborne Pathogens and Disease, 14*, 302–307.

Moon, K. D., Delaquis, P., Toivonen, P., & Stanich, K. (2006). Effect of vanillin on the fate of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in a model apple juice medium and in apple juice. *Food Microbiology, 23*, 169–174.

Nebot Sanz, E., Salcedo Dávila, I., Andrade Balao, J. A., & Quiroga Alonso, J. M. (2007). Modelling of reactivation after UV disinfection: Effect of UV-C dose on subsequent photoreactivation and dark repair. *Water Research, 41*, 3141–3151.

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International Journal of Food Microbiology, 156*, 7–17.

Ochoa-Velasco, C. E., Díaz-Lima, M. C., Ávila-Sosa, R., Ruiz-López, I. I., Corona-Jiménez, E., Hernández-Carranza, P., et al. (2018). Effect of UV-C light on *Lactobacillus rhamnosus*, *Salmonella* Typhimurium, and *Saccharomyces cerevisiae* kinetics in inoculated coconut water: Survival and residual effect. *Journal of Food Engineering, 223*, 255–261.

Prades, A., Dornier, M., Diop, N., & Pain, J. P. (2012a). Coconut water uses, composition and properties: A review. *Fruits, 67*, 87–107.

Prades, A., Dornier, M., Diop, N., & Pain, J. P. (2012b). Coconut water preservation and processing: A review. *Fruits, 67*, 157–171.

Preetha, P. P., Devi, V. G., & Rajamohan, T. (2012). Hypoglicemic and antioxidant potential of coconut water in experimental diabetes. *Food & Function, 3*, 753–757.

Rastogi, R. P., Richa, Kumar, A., Tyagi, M. B., & Sinha, R. P. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids, 2010*, 1–32.

Rupasinghe, H. P. V., Boulter-Bitzer, J., Ahn, T., & Odumeru, J. A. (2006). Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples. *Food Research International, 39*, 575–580.

Samant, S. S., Crandall, P. G., ÓBryan, C., Lingbeck, J. M., Martin, E. M., & Seo, H. S. (2015). Sensory impact of chemical and natural antimicrobials on poultry products: A review. *Poultry Science, 94*, 1–12.

Unluturk, S., Atilgan, M. R., Baysal, A. H., & Tari, C. (2008). Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). *Journal of Food Engineering, 85*, 561–568.

Wendakoon, C. N., & Sakaguchi, M. (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. *Journal of Food Protection, 58*, 280–283.

Yin, X., Goudriaan, J., Lantinga, E. A., Vos, J., & Spiertz, H. J. (2003). A flexible sigmoid of determinate growth. *Annals of Botany, 91*, 361–371.

Yong, J., Ge, L., Ng, Y., & Tan, S. N. (2009). The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. *Molecules, 14*, 5144–5164.

Zhou, F., Ji, B., Zhang, H., Jiang, H., Yang, Z., Li, J., et al. (2007). The antibacterial effect of cinnamaldehyde, thymol, carvacrol and their combinations against the foodborne pathogen *Salmonella* Typhimurium. *Journal of Food Safety, 27*, 124–133.