



Kinetics model of microbial degradation by UV radiation and shelf life of coconut water



Sirirat Donsingha^a, Kitipong Assatarakul^{a, b, *}

^a Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

^b Special Task Force of Activating Research (STAR) in Novel Technology for Food Packaging and Control of Shelf Life, Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

ARTICLE INFO

Article history:

Received 18 February 2018

Received in revised form

10 April 2018

Accepted 15 April 2018

Available online 18 April 2018

Keywords:

Ultraviolet radiation

Inactivation kinetics

Shelf life

Coconut water

ABSTRACT

The aim of this present study was to investigate the effect of UV radiation on microbial inactivation kinetic, quality and shelf life of coconut water. Zero-order and first-order kinetic models were used to investigate microbial degradation (*Escherichia coli* O157:H7, *Staphylococcus aureus*, *Salmonella* Enteritidis, and *Lactobacillus plantarum*) by UV radiation (0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 4.8, 8.0 and 12.0 J/mL). UV dose of 1.6, 3.2 and 4.8 J/mL was applied to investigate the effect of UV radiation on physical properties (pH, °Brix, color and turbidity), chemical properties (total phenolic compound or TP and polyphenol oxidase or PPO activity) of coconut water and to investigate the shelf life of coconut water by determination of pink discoloration, total plate count (TPC), and yeast and mold count (YMC) during cold storage at 4 °C for 18 days compared to pasteurized sample (95 °C 100 s). Results showed that microbial inactivation of all bacteria tested in this study followed first-order kinetic model according to higher coefficient of determination (0.9115–0.9656). *E. coli* O157:H7 was found to be the most sensitive bacteria to UV radiation with regard to highest population reduction in coconut water. In addition, no significant changes were detected in the investigation of L^* , b^* , pH, °Brix, titratable acidity, and turbidity of coconut water treated with UV radiation ($p > 0.05$). However, UV treatment showed significant effect on a^* , TP and PPO activity ($p \leq 0.05$). TPC of all samples increased while YMC decreased during storage at 4 °C. According to microbial investigation during storage at 4 °C, it was found that shelf life of fresh coconut water (control) was 4 days and increased to 6, 10 and 16 days for samples treated with UV dose of 1.6, 3.2 and 4.8 J/mL, respectively. These results of this study support the application of UV radiation in coconut water to preserve quality and prolong shelf life in conjunction with safety aspects as an alternative method to thermal processing.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Over the last decades, the consumer's demand of minimally-processed foods or fresh-like products has considerably increased. Generally, thermal processing is a conventional method to ensure the safety and prolong the shelf life of juice products. However, heat treatment can also cause detrimental effect on physical, chemical, nutritional and sensory properties of juice product. In order to preserve quality of juice, considerable efforts have been directed towards the application of novel non-thermal processing. Therefore, emerging non-thermal technology which is

defined as a technology that uses mechanisms other than conventional heat treatment to inactivate both pathogen and spoilage microorganisms has received increasing attention in recent years. Its application improves safety and quality and has become an alternative method to guarantee the product's safety while the degradation quality is minimized (Huang, Wu, Lu, Shyu, & Wang, 2017).

Ultraviolet radiation (UV) is one of the most interesting non-thermal processing methods due to its several advantages. UV is able to inactivate a wide range of pathogenic and spoilage microorganisms in juices (Gabriel, 2012). The germicidal effect of UV radiation is due to the absorption of UV photon by microbial deoxy nucleic acid (DNA) which results in cross-linkage of two pyrimidine bases; resulting in the prevention of DNA transcription and replication and eventually causing cell death. Last few decades, UV has

* Corresponding author. Department of Food Technology, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand.

E-mail address: Kitipong.A@chula.ac.th (K. Assatarakul).

been successfully used as disinfectant method for air, surfaces and drinking water (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Bolton, 2010; Koutchma, 2009). In 2000, UV has been approved by USFDA as alternative pasteurization method for juice and beverage in the case of at least 5-log reduction of target microorganisms; commonly pathogen, under turbulent flow conditions (USFDA, 2012).

Coconut water is one of the most popular beverages in tropical countries with delicate and unique flavor. Coconut water, a clear liquid from coconut fruit, is regarded as a healthy drink as it is rich in several minerals (calcium, magnesium and potassium), vitamins (vitamin B and vitamin C), enzyme with anti-inflammatory properties and antioxidants (DebMandal, & Mandal, 2011). Based on FAO report, it is claimed as a natural alternative to sport drinks with low calories (FAO, 2000). According to World Health Organization (WHO), it is suggested that consumption of coconut water is a rehydration remedy in case of acute diarrhea (WHO, 2009). As a result, market opportunities have been established with increasing demand, especially for freshly-prepared coconut water. Nowadays, coconut water is commercially processed by heat treatment to inactivate natural microflora normally found in this product and in order to extend the shelf life of the product. However, the high temperature greatly alters the sensory attributes and nutritional properties. In addition to spoilage microorganisms, different foodborne outbreaks associated with coconut water have been internationally reported including *Staphylococcus aureus* and *Escherichia coli* under refrigeration (Hoffmann, Coelho, Mansor, Takahashi, & Vinturim, 2002; Melo, Cardonha, & Oliveira, 2003; Strawn, Schneider, & Danyluk, 2011). Therefore, to delay the spoilage and to guarantee the safety of coconut water without alteration in nutritional and sensory quality have become a challenge for the industry.

Besides microbial spoilage and foodborne pathogens, biochemical reaction by inherent enzyme such as polyphenol oxidase (PPO) could play an important role in juice's shelf life and consumer's acceptance and safety. PPO, a copper containing enzyme, is naturally found in many fruits and it catalyzes oxidation reaction of phenolic compounds which lead to the polymerization resulting in unpleasant brown pigments (Chisari et al., 2001). While the inactivation of spoilage and pathogen in fruit juices is well documented, only little published work on the effects of UV radiation on this enzyme in fruit juices is available to date. Therefore, the objectives of this study were to investigate the effect of UV radiation on kinetics of microbial inactivation and quality of coconut water and to determine the effect of UV radiation on shelf life of coconut water during storage at 4 °C.

2. Materials and methods

Coconut water preparation: Coconut water was obtained from Ocha Food Pack company (Bangkok, Thailand) and coconut water did not contain any preservatives. Fresh coconut water was prepared and stored at 4 °C before immediate use for experiment.

Bacterial strains and media: The selection of bacterial strains in this study was based on the potential cause of foodborne outbreak and food spoilage in coconut water. Bacterial strains of *E. coli* O157:H7 DMST 12743, *S. aureus* DMST 4745 and *Salmonella* Enteritidis DMST 15676 were obtained from the Department of Medical Sciences (Bangkok, Thailand) whereas *Lactobacillus plantarum* TISTR 875 was obtained from the Thailand Institute of Scientific and Technological Research. These cultures were kept in tryptic soy agar (TSA; Difco, USA) and each strain was maintained in 20% (w/v) glycerol at -40 °C. For microbial degradation kinetics experiment, *E. coli* O157:H7, *S. aureus* and *Salmonella* Enteritidis were enumerated in tryptic soy broth (Difco, USA) while *L. plantarum* was

enumerated in MRS broth (Difco, USA) at 37 °C for 24 h. Each culture was transferred into freshly-prepared coconut water in sterile flask to achieve final microbial concentration of 10^7 – 10^8 CFU/mL.

3. Experiment on microbial degradation kinetics

UV treatments were carried out in a UV unit as showed in Fig. 1. Germicidal UV lamp (8 watt) was used with peak emission at 254 nm and UV lamp was stabilized by being switched on for 10 min.

Inoculated coconut water was subjected to UV unit to achieve the final UV dose of 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 4.8, 8.0 and 12.0 J/mL. Appropriate dilution was made with 0.85% NaCl and pour plate technique was used to enumerate the survival bacteria (AOAC, 1995). Nutrient agar (Difco, USA) was used to grow *E. coli* O157:H7, *S. aureus*, and *Salmonella* Enteritidis whereas MRS agar was used for *L. plantarum*. Plates were incubated at 37 °C for 48 h. Counts were performed in duplicate and data were plotted according to zero-order and first-order kinetic models as showed in equation (1) and equation (2), respectively.

$$[N] = [N_0] - kt \quad (1)$$

$$[N] = [N_0] e^{-kt} \text{ or } \ln([N]/[N_0]) = -kt \quad (2)$$

where N_0 is initial number of microorganisms (log CFU/mL), N is number of surviving microorganisms (log CFU/mL), k is a rate constant and t is UV dose (J/mL). The most fitted model was selected based on coefficient of determination (R^2) calculated using the least square method.

4. Comparison of UV and pasteurized treatments on physical and chemical properties, pink discoloration and shelf life of coconut water

Uninoculated coconut water was used in this experiment and samples were subjected to UV radiation (1.6, 3.2 and 4.8 J/mL) compared to pasteurized treatment (95 °C 100 s). Physical properties (pH, °Brix, color values, turbidity) and chemical properties (TP, PPO activity) of treated samples were investigated and pink discoloration, total plate count (TPC) and yeast and mold count (YMC) of samples were determined during storage at 4 °C for 18 days.

5. Physical properties of coconut water

Total soluble solid (°Brix) was measured by hand refractometer (Atago, Master- α , USA) while pH was measured with pH meter (Mettler Toledo, FEP20 FiveEasy Plus™, Switzerland). Color values (L^* , a^* , b^*) were determined by CIE color system using a Chroma Meter (Minolta CR 400, Konica Inc., Japan) and total color difference (ΔE) was calculated by equation (3)

$$\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \quad (3)$$

where L is the lightness of sample, L_0^* is lightness of control, a^* is the redness of sample, a_0^* is the redness of control, b^* is the yellowness of sample and b_0^* is the yellowness of control. Titratable acidity was determined by titration with 0.1 N NaOH and calculated as % malic acid (AOAC, 2000). Turbidity was measured as a degree of light scattering by particles using a UV-spectrophotometer (Thermo Spectronic, GENESYS 10 UV, USA) at wavelength of 610 nm according to the method described by Tan, Cheng, Bhat, Rusul, and Easa (2014).

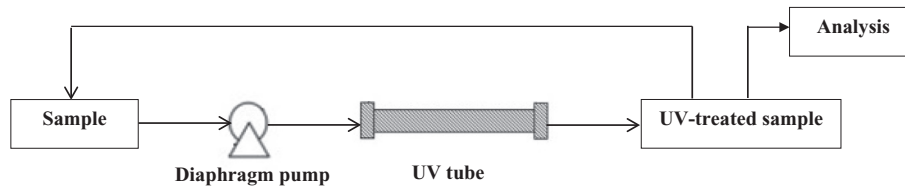


Fig. 1. UV unit diagram.

6. Chemical properties of coconut water

Total phenolic compound (TP) of sample was determined by Folin-Ciocalteu method with some modification (Tan et al., 2014). The reaction mixture included 0.1 mL sample, 7 mL distilled water and 0.5 mL Folin-Ciocalteu reagent. The mixture then incubated at room temperature for 5 min before adding 1.5 mL of 7.5% w/v sodium carbonate and added distilled water until the total volume was 10 mL in volumetric flask. The mixture then incubated at room temperature for 2 h and the absorbance of sample was measured at 760 nm using a UV-spectrophotometer with distilled water as a blank. Gallic acid (Sigma-Aldrich, USA) was used to prepare standard curve for total phenolic content determination and the result was expressed as μg gallic acid equivalent per milliliter of sample.

7. Polyphenol oxidase (PPO) activity

PPO activity was determined according to the method described by Tan et al. (2014) with slight modification. The reaction mixture contained 5.5 mL of 0.2 M monobasic sodium phosphate/dibasic sodium phosphate pH 6.0 and 1.5 mL of 0.2 M pyrocatechol and then left the mixture in water bath until the stabilization of temperature at 25 °C. Then, 2 mL of sample were added to the mixture and absorbance of sample was immediately measured at the wavelength 425 nm using a UV-spectrophotometer. Blank solution was prepared by mixing 1.5 mL of 0.2 M pyrocatechol and 5.5 mL phosphate buffer. The absorbance value was determined every 30 s for 30 min. Then absorbance value was plotted against time and initial slope was used to calculate PPO activity. Enzyme activity unit was expressed as an amount of enzymatic extract used to increase absorbance at rate of 0.001 unit per mL of sample per total soluble solid content per minute ($\text{U}/\text{mL} \cdot \text{Brix min}$).

8. Pink discoloration

Twenty five samples of each treatment (250 mL each sample) were used for pink discoloration experiment. Samples were kept at 4 °C for 18 days and the pink discoloration was checked every 2 days. Pink color evaluation was carried out by rating the pink color in the hedonic rating score from 1 to 5 (1 = no pink color and 5 = pink color) as showed in Fig. 2. Sample with score 5 only that was accounted for pink discoloration calculation and percent discoloration was calculated by equation (4)

$$\% \text{ pink discoloration} = N_{\text{pink}}/N_{\text{total}} \times 100 \quad (4)$$

where N_{pink} is number of pink sample and N_{total} is number of samples (25 samples).

9. Total plate count (TPC) and yeast and mold count (YMC)

TPC and YMC were investigated on plate count agar (Difco, USA) and potato dextrose agar (Difco, USA), respectively (AOAC, 1995). Plates were incubated at 37 °C for 48 h and 30 °C for 72 h for TPC

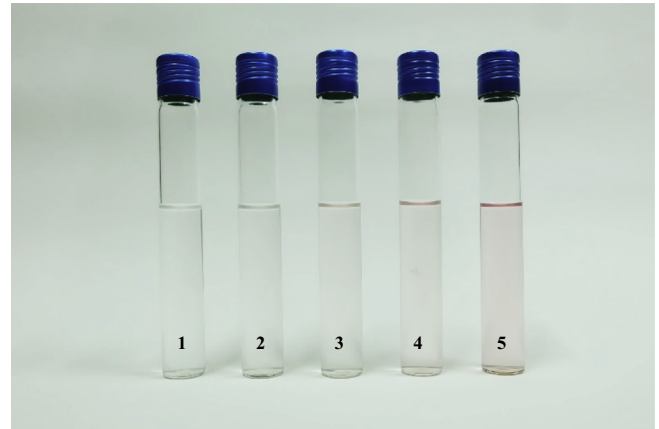


Fig. 2. Pink discoloration score of coconut water (score 1–5).

and YMC, respectively. Results were calculated and expressed as colony forming units/mL (CFU/mL).

10. Statistical analysis

All experiments were conducted in triplicate and kinetic parameters were calculated by Microsoft Excel 2010. Results were expressed as mean \pm standard deviation (SD). Analysis of variance of data was carried out using Statistical Package for the Social Sciences (SPSS) 17 (SPSS Statistical Software, Inc., Chicago, IL, USA) and significant differences between mean values were determined by Tukey's honest significant difference (HSD) test at a significance level of $\alpha = 0.05$.

11. Results and discussion

Kinetics modeling is a useful tool in relation to food processing and food quality. Changes of foods due to the processing and storage condition cause alteration in food quality including physical, chemical, biochemical and microbiological properties and these changes can be explained by kinetics modeling. It is also a powerful technique to reveal fundamental reaction mechanisms and assists in the management of quality control. Zero-order and first-order kinetic models are a common approach to investigate microbial inactivation in food product (Van Boekel, 1998).

Zero-order and first-order kinetic models were graphically plotted with population scale (primary Y-axis) and \ln (population) scale (secondary Y-axis), respectively, as showed in Fig. 3. Based on kinetics comparison by regression analysis, it was found that the microbial degradation agrees better with first-order kinetic model due to the higher coefficient of determination (R^2) compared to zero-order kinetic model (Table 1). Coefficient of determination ranged from 0.2085 to 0.2937 and from 0.9115 to 0.9656 for zero-order and first-order kinetic models, respectively while the rate constants (k), a reaction rate, were between 148705 and 1000000

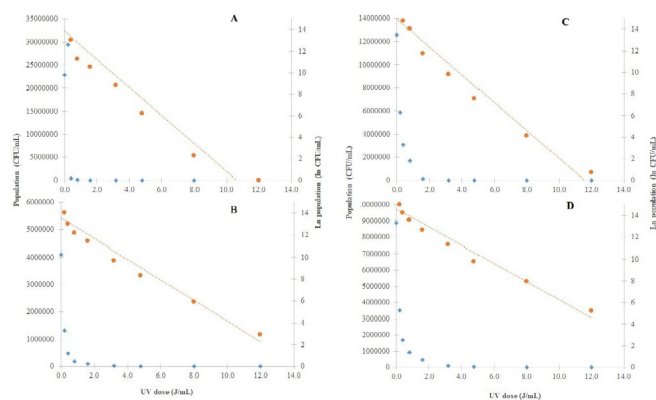


Fig. 3. Modeling of degradation kinetics: plot describing zero-order (◆) and first-order (●) inactivation of *Escherichia coli* O157:H7 (A), *Salmonella* Enteritidis (B), *Staphylococcus aureus* (C) and *Lactobacillus plantarum* (D) treated with UV radiation.

and between 0.8320 and 1.3021 for zero-order and first-order kinetic models, respectively. These results are in agreement with Bhullar and others (2018) who studied the microbial inactivation kinetics of coconut water by UV radiation and it was found that degradation kinetic models of *E. coli*, *Salmonella* Typhimurium and *Listeria monocytogenes* caused by UV radiation followed first-order kinetic model. Moreover, the report by Tran and Farid (2004) showed that microbial reduction of aerobic plate count (APC) and yeast and mold count (YMC) caused by UV radiation in orange juice fitted first-order kinetic model with R^2 of 0.98 and 0.93 for APC and YMC, respectively.

The results of this study showed that *E. coli* O157:H7 was the most sensitive bacteria to UV radiation due to the highest k while the most resistant bacteria was *L. plantarum*. Gram negative bacteria are generally more sensitive to UV radiation than Gram positive bacteria due to the ability to repair damaged-DNA caused by UV radiation (Arrage, Phelps, Benoit, & White, 1993). *L. plantarum*, lactic acid bacteria, are Gram positive, non-spore forming and fermentative bacteria that are naturally found in coconut water and they can be used as a spoilage indicator of coconut water (Prado et al., 2015).

There have been a number of cases associated with pathogenic *E. coli* contamination in juice and it showed the ability to grow in acidic condition such as apple juice (Splittsoesser et al., 1994). Besides *E. coli* specified by legislation, *S. aureus* is also important due to its relationship with the hygienic conditions of handling and processing. *S. aureus* is one of the major cause of foodborne disease throughout the world. It is naturally found on skin, nail and hair of human and the most important route of *S. aureus* contamination in food is unhygienic handling (Asperger & Zangerl, 2002). With regard to safety concern, inhibition of *E. coli* and *S. aureus* should be necessarily maximized to satisfy consumer's expectation.

There were no significant differences in L^* , b^* , pH, °Brix, % titratable acidity (% malic acid) and turbidity ($p > 0.05$) for treated

samples (Table 2). Results are in agreement with previous studies that reported no significant differences in physicochemical properties (pH, °Brix, % titratable acidity) were found in juices (Noci et al., 2008; Pala & Toklucu, 2013).

Phenolic compounds are secondary metabolites naturally found in fruits and vegetables and their beneficial effects have been related to antioxidant activity. Phenolic compounds are considered as the most important antioxidant in fruit and vegetable products; therefore, fruit and vegetable including their product such as fruit juice are natural sources of antioxidants (Balasundram, Sundram, & Samman, 2006). The results demonstrated the significant effect of UV radiation and thermal pasteurization on TP. Compared to control the level of TP has significantly ($p \leq 0.05$) decreased in coconut water treated by UV radiation and pasteurization. However, degradation of TP in UV samples was significantly lower than pasteurized sample. Retaining TP in coconut water would be a great health benefit since TP provides antioxidant activity. Results from this present study are in agreement with the study from Noci et al. (2008) who reported that phenolic compound significantly decreased in apple juice treated with UV radiation compared to control. The mechanism of degradation in phenolic compound is due to the photooxidation reaction that significantly increases the alteration in phenolic compound (Koutchma, Forney, & Moraru, 2009). Moreover, thermal processing can cause phenolic compound to be degraded by structure alteration in fruit juice (Noci et al., 2008).

Color is a key consideration perceived by consumer in quality assessments of fruits, fruit juices and fruit products (Khan, Tango, Miskeen, Lee, & Oh, 2017). Results showed that there were no significant differences between mean values of L^* and b^* of samples; however, significant differences were found between mean values of a^* of samples. In addition, a^* and ΔE increased with increasing UV dose meaning that UV could affect the redness of coconut water and pasteurized sample had the highest a^* and ΔE which mean that pasteurization could accelerate the pink discoloration in coconut water. Pink discoloration caused by UV radiation and pasteurization might be an adverse effect in coconut water since clear and white color of coconut water are consumer's perception. According to the report of Cserhalmi, Sass-Kiss, Tóth-Markus, and Lechner (2006), ΔE can be divided in 5 categories as follows 1) not observable ($0 < \Delta E < 0.5$), slightly observable ($0.5 < \Delta E < 1.5$), observable ($1.5 < \Delta E < 3.0$), well observable ($3.0 < \Delta E < 6.0$) and greatly observable ($6.0 < \Delta E < 12$). Sample treated with UV 3.2 and 4.8 J/mL and pasteurized samples can be slightly observable due to the fact that ΔE was between 0.5 and 1.5.

Pink discoloration in treated samples during the storage at 4 °C is shown in Fig. 4. Pasteurized sample showed 100% pink discoloration on day 6 while UV treated sample with 4.8, 3.2 and 1.6 J/mL showed 100% pink discoloration on day 8, 10 and 18, respectively. Therefore, UV dose at 4.8, 3.2 and 1.6 J/mL delayed the occurrence of pink discoloration by 2, 4 and 12 days, respectively. Incidence of pink discoloration in coconut water was a consequence of PPO activity and the oxidation of phenolic compound with manganese and a catalyst (Choeisunthon & Tongchitpakdee, 2013). Moreover,

Table 1

Rate constant (k) and coefficient of determination (R^2) of zero-order and first-order kinetic models of microbial degradation by UV radiation in coconut water.

Microorganisms	Zero-order kinetic model		First-order kinetic model	
	Rate constant	Coefficient of determination	Rate constant	Coefficient of determination
	(k)	(R^2)	(k)	(R^2)
<i>E. coli</i> O157:H7	1000000	0.2085	1.3021	0.9115
<i>Salmonella</i> Enteritidis	148705	0.2106	0.9402	0.9555
<i>S. aureus</i>	554565	0.2937	1.2876	0.9656
<i>L. plantarum</i>	364888	0.2703	0.8320	0.9576

Table 2
Physical and chemical properties of coconut water treated with UV radiation.

Treatment	pH ^{ns}	°Brix ^{ns}	Titratable acidity ^{ns}		Turbidity ^{ns}	TP	PPO activity	Color			ΔE
			(% malic acid)					($\mu\text{g GAE/mL}$) [*]	(U/mL °Brix min)	L^{*ns}	
control	5.47 ± 0.10	6.83 ± 0.29	0.08 ± 0.01		5.74 ± 0.49	82.59 ^a ± 5.34	0.0263 ^a ± 0.0014	37.71 ± 3.40	0.61 ^b ± 0.02	1.82 ± 2.39	0.00
UV 1.6 J/mL	5.42 ± 0.17	6.63 ± 0.15	0.08 ± 0.03		5.72 ± 0.48	64.08 ^b ± 7.58	0.0237 ^{ab} ± 0.0008	37.67 ± 3.23	0.62 ^b ± 0.03	1.97 ± 2.48	0.30 ± .018
UV 3.2 J/mL	5.41 ± 0.27	6.93 ± 0.42	0.07 ± 0.02		5.72 ± 0.44	61.67 ^b ± 1.47	0.0225 ^b ± 0.0025	37.82 ± 2.97	0.67 ^b ± 0.09	1.95 ± 2.43	0.66 ± 0.50
UV 4.8 J/mL	5.37 ± 0.26	6.80 ± 0.20	0.08 ± 0.03		5.76 ± 0.56	61.30 ^b ± 3.06	0.0141 ^c ± 0.0015	37.11 ± 3.27	0.75 ^b ± 0.04	2.12 ± 2.228	0.89 ± 0.62
Pasteurization	5.42 ± 0.25	6.93 ± 0.31	0.08 ± 0.03		5.73 ± 0.52	44.26 ^c ± 7.08	0.0078 ^d ± 0.0008	37.65 ± 3.07	0.96 ^a ± 0.08	2.08 ± 2.53	1.14 ± 0.68

Results are presented as “mean ± standard deviation” Least significant difference was determined by Tukey’s HSD test at $\alpha = 0.05$.

ns: no significant differences between the mean values ($\alpha = 0.05$) within the column.

a,b,c.: the different letters in same column indicate significant differences ($p \leq 0.05$).

* $\mu\text{g GAE/mL} = \mu\text{g gallic acid equivalent/mL}$.

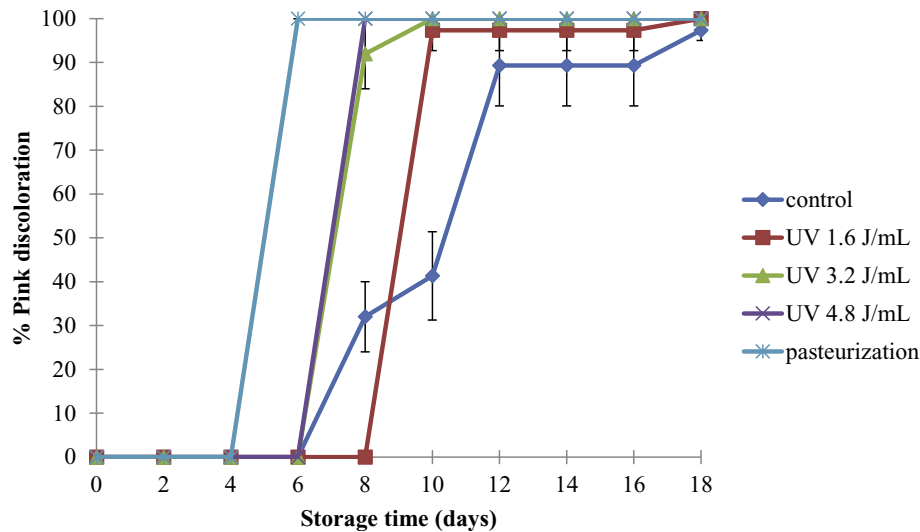


Fig. 4. Pink discoloration (%) of coconut water treated with UV radiation during storage at 4 °C.

stage of maturation of coconut could affect pink discoloration and it has been reported that PPO activity was completely inactivated by UV after 100 min exposure at 45 °C while 60% reduction in PPO activity was achieved after 120 min exposure at 25 °C in clarified nectarine juices (Aguilar, Ibarz, Garvín, & Ibarz, 2016). Table 2 shows PPO activity in the coconut water and it can be seen that the higher in UV radiation, the greater was the reduction on PPO activity. This result is in accordance with Müller, Noack, Greiner, Stahl, and Posten (2014) who reported that UV (0–100.48 kJ/L) significantly affected PPO activity and PPO activity was reduced to 44.3% in apple juice after UV treatment of 60.29 kJ/L. Moreover, UV treatment completely inactivated PPO activity after 100 min treatment with minor changes in physicochemical properties of nectarine juices (Aguilar et al., 2016). Inactivation of PPO activity by UV radiation could be a consequence of protein aggregation. UV photon could be absorbed by protein which causes a protein structure modification and therefore leads to an alteration in enzyme activity (Manzocco, Quarta, & Dri, 2009).

Changes in TPC and YMC during cold storage at 4 °C are shown in Fig. 5 and Fig. 6, respectively. During the storage at 4 °C, TPC increased and YMC decreased in all samples. According to Institute of Food Science and Technology (IFST), the maximum acceptable numbers of TPC and YMC in fruit juice are 4 log CFU/mL and 3 log CFU/mL, respectively (IFST, 1993). The shelf life of UV-treated coconut water was extended to 2, 6 and 12 days for UV treatments of 1.6, 3.2 and 4.8 J/mL, respectively, compared to control (untreated coconut water). UV increased shelf life of coconut water approximately 1.5–4 times. Regarding pasteurized sample and UV-treated

sample (4.8 J/mL) stored at 4 °C, number of YMC reached undetectable level in day 0 and day 4, respectively. It is due to the fact that yeast and mold tend to be more sensitive to heat than bacteria and they are typically destroyed by heat treatment at temperature of 60–71 °C which is a general condition of pasteurization (Fleming & Costilow, 2004). In addition, bacteria are more sensitive to UV than yeast and mold because bacteria are smaller which is easier for UV passage and higher composition of pyrimidine bases (cytosine and tyrosine) of bacterial DNA compared to yeast and mold results in a higher in a cross linkage which leads to preventing cell replication (Miller, Jeffrey, Mitchell, & Elasri, 1999).

Coconut water is initially sterile and remains aseptic as long as fruit injury has not occurred that allows the entrance for microorganisms. The microbiology of coconut water depends on the microorganisms considered as a target microorganism and is a challenge from safety and product stability perspective. The specific pH and high concentration of sugar in coconut water are influential factors that promote the growth of lactic acid bacteria (LAB) and consequently trigger a spoilage. LAB was reported as the most important group of spoilage microorganism in fruit juice especially genera of *Lactobacillus* (Dharmasena, Barron, Fraser, & Jiang, 2015). In addition, yeasts are able to grow under low pH, high sugar content condition and refrigeration temperature resulting in a potential spoilage microorganism in juice and coconut water (Tribst, Sant’Ana, & Massaguier, 2009). Yeast spoilage can be detected by producing carbon dioxide and alcohol, increasing turbidity, leading to flocculation and phase separation as a result of enzymatic reaction on pectin (Gokmen & Acar, 2004).

- 400B. *Foods*, 4(3), 328–337.
- Fleming, H. P., & Costilow, R. N. (2004). *Acidified Foods: Principles of handling and preservation*. St. Charles, IL: Pickle packers international Inc. Food and agricultural organization (FAO), (2000). New sports Drink: Coconut water (Accessed on October, 2017) <http://www.fao.org/ag/magazine/9810/spot3.htm>.
- Gabriel, A. A. (2012). Inactivation of *Escherichia coli* O157:H7 and spoilage yeasts in germicidal UV-C-irradiated and heat-treated clear apple juice. *Food Control*, 25(2), 425–432.
- Gokmen, V., & Acar, J. (2004). Fumaric acid in apple juice: A potential indicator of microbial spoilage of apples used as raw material. *Food Additives & Contaminants*, 21, 626–631.
- Hoffmann, F. L., Coelho, A. R., Mansor, A. P., Takahashi, C. M., & Vinturim, T. M. (2002). Qualidade microbiológica de amostras de água de coco vendidas por ambulantes na cidade de São João do Rio Preto – SP. *Revista Higiene Alimentar*, 16, 87–92.
- Huang, H. W., Wu, S. J., Lu, J. K., Shyu, Y. T., & Wang, C. Y. (2017). Current status and future trends of high-pressure processing in food industry. *Food Control*, 72, 1–8.
- Institute of Food Science & Technology (IFST). (1993). *Shelf life of foods: Guidelines for its determination and prediction*. London: Institute of Food Science & Technology (UK).
- Khan, I., Tango, C. N., Miskeen, S., Lee, B. H., & Oh, D. H. (2017). Hurdle technology: A novel approach for enhanced food quality and safety – a review. *Food Control*, 73, 1426–1444.
- Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food and Bioprocess Technology*, 2(2), 138–155.
- Koutchma, T., Forney, L. J., & Moraru, C. I. (2009). *Ultraviolet light in food Technology: Principles and applications*. New York: CRC Press.
- Manzocco, L., Quarta, B., & Dri, A. (2009). Polyphenoloxidase inactivation by light exposure in model systems and apple derivatives. *Innovative Food Science & Emerging Technologies*, 10(4), 506–511.
- Melo, N. P. M., Cardonha, A. M. S., & Oliveira, A. C. F. (2003). Qualidade microbiológica das águas de coco envasadas e comercializadas na cidade de Natal – RN. *Revista Higiene Alimentar*, 17, 113–114.
- Miller, R., Jeffrey, W., Mitchell, D., & Elasri, M. (1999). Bacterial responses to ultraviolet light. *American Society for Microbiology*, 65, 535–541.
- Müller, A., Noack, L., Greiner, R., Stahl, M. R., & Posten, C. (2014). Effect of UV-C and UV-B treatment on polyphenol oxidase activity and shelf life of apple and grape juices. *Innovative Food Science & Emerging Technologies*, 26, 498–504.
- Noci, F., Riemer, J., Walkling-Ribeiro, M., Cronin, D., Morgan, D., & Lyng, J. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering*, 85(1), 141–146.
- Pala, C. U., & Toklucu, A. K. (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*, 50(2), 426–431.
- Prado, F. C., Lindner, J. D. D., Inaba, J., Thomaz-Soccol, V., Brar, S. K., & Soccol, C. R. (2015). Development and evaluation of a fermented coconut water beverage with potential health benefits. *Journal of Functional Foods*, 12, 489–497.
- Splittstoesser, D. F., Churey, J. J., & Lee, C. Y. (1994). Growth characteristics of aciduric sporeforming bacilli isolated from fruit juices. *Journal of Food Protection*, 57, 1080–1083.
- Strawn, L. K., Schneider, K. R., & Danyluk, M. D. (2011). Microbial safety of tropical fruits. *Critical Reviews in Food Science and Nutrition*, 51, 132–145.
- Tan, T. C., Cheng, L. H., Bhat, R., Rusul, G., & Easa, A. M. (2014). Composition, physicochemical properties and thermal inactivation kinetics of polyphenol oxidase and peroxidase from coconut (*Cocos nucifera*) water obtained from immature, mature and overly-mature coconut. *Food Chemistry*, 142, 121–128.
- Tran, M. T. T., & Farid, M. (2004). Ultraviolet treatment of orange juice. *Innovative Food Science & Emerging Technologies*, 5(4), 495–502.
- Tribst, A. A. L., Sant'Ana, A. S., & Massaguier, P. R. (2009). Review: Microbiological quality and safety of fruit juices - past, present and future perspectives. *Critical Reviews in Microbiology*, 35(4), 310–339.
- United States Food and Drug Administration (USFDA). (2012). Irradiation in the production, processing, and handling of food. Final rule. *Federal Register*, 77(231), 71316–71320.
- Van Boekel, M. A. J. S. (1998). Modelling of chemical reactions in foods: A multi-response approach. *Acta Horticulturae*, 476, 149–155.
- World Health Organization (WHO). (2009). Diarrhoea: Why children are still dying and what can be done (Accessed on September, 2017) http://apps.who.int/iris/bitstream/10665/44174/1/9789241598415_eng.pdf.