

Simultaneous UV-C and Ultrasonic Energy Treatment for Disinfection of Tomatoes and Its Antioxidant Properties

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Abstract—The simultaneous use of UV-C radiation at 640 or 900 μWcm^{-2} and ultrasonic energy of 13.78 W/L at 40 kHz was applied on tomatoes to study its ability to disinfect tomatoes from microbes. Total aerobic bacteria and yeast and mold population decreased with increase of UV-C dosage application from 0.72-10.76 kJ/m^2 at constant ultrasonic energy supply. At simultaneous treatment dosage of 6.46 kJ/m^2 , total aerobic bacteria were significantly ($p < 0.001$) reduced by 2 log reductions. Yeast and mold survivors were undetected at higher dosage treatment from 4.31 to 10.76 kJ/m^2 . Lower dosage treatment from 0.72 to 2.15 kJ/m^2 presented minor log reductions of 0.14 to 0.75. The treatment also stimulated 41.26-50.37% increase of total phenols from an initial value of 13.38 mg GAE 100g⁻¹ FW. Antioxidant activity increase from 27.51-36.07% was obtained at dosage level of 8.61 kJ/m^2 .

Index Terms—total aerobic bacteria, yeast and mold, total phenolic content, antioxidant activity, hydrophilic extract

I. INTRODUCTION

Tomato is one of the world's most commonly consumed vegetable [1] and an essential component of the Mediterranean diet regularly used as food due to its notable and abundant array of antioxidant micronutrients content necessary for healthy living [2], [3]. Being climacteric with high perishability and life span usually 2 to 3 weeks, extending their shelf life is a very significant consideration for both exports and domestic markets [1]. Losses arise from tomato spoilage which are as high as 30-50% in tropical and developing countries and occur in the form physical losses and losses in minerals, essential nutrients and vitamins [4], [5]. Spoilage is usually due to disease infestation and it is high during the wet and warm climates as those temperatures encourage progression of spoilage and pathogenic organisms [6]. Series of disease outbreaks have been linked to tomato, with *Salmonella* as the most common bacterial pathogen [7]. Tomato is preferred substrate for the bacterial pathogen *Salmonella*, with varied serotypes and contamination sources within

different outbreaks [8], [9]. Reference [10] reported 2 different tomato associated outbreaks linked to Norovirus and *Salmonella* spp. in Europe and 1 each linked to Hepatitis A virus and *Campylobacter* spp., 5 and 17 outbreaks linked to Norovirus and *Salmonella* spp. respectively in the United States of America within 2004 and 2012.

The ultraviolet-C (UV-C) radiation and ultrasonic energy have been used as new approaches with promising potential for shelf life extension, decontamination, microbial load reduction and improvement of nutritional and organoleptic properties of tomato [11]-[13]. They are continuously being evaluated as alternatives to conventional thermal processes and the use of sanitizers. Single or combined treatments is used in tomato postharvest handling to prevent spoilage, decay and inactivate microbes. They include washing, hot and warm water treatments, organic acids, thermal destruction, electrolyzed oxidising water, chlorinated water, edible coatings, ozonated water, modified atmosphere packaging, low temperature storage, irradiation, ultraviolet radiation, ultrasound technology, high pressure processing and pulsed electric field [14]-[16]. This research investigated the synergistic effect of simultaneous use of UV-C radiation and ultrasonic energy on disinfecting tomatoes in terms of microbial load reduction as studies reported earlier were either single treatment or sequential combination. The functional properties of tomatoes of total phenols and antioxidant activity were also measured since they are quality indicators of the fruit.

II. MATERIALS AND METHODS

A. Sample Preparation

Mature tomato fruits (*Solanum lycopersicum* cv. Baby TM1536) were manually harvested at the turning stage, i.e. between 10 to 30% surface aggregate showing a definite change in colour from green to tannish-yellow, pink, red or a combination of both from a commercial farm in Cameron Highland District, Pahang, Malaysia (4.5971° N, 101.4160° E). The tomatoes were screened

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for uniform shapes and free from cuts, bruises and infections for the experiment. Selected samples were transported to the laboratory and stored in a cold room at $12 \pm 2^\circ\text{C}$ and relative humidity of $76 \pm 2\%$ without any form of processing until treatment. Storage temperature choice was based on reports from literature for tomato storage [17], [18], especially temperatures that support higher rates of biosynthesis of some organic compounds in tomato [19].

B. Disinfecting Equipment

The disinfecting equipment was a laboratory scale stainless steel tank with dimension 406 mm (L) x 406 mm (W) x 610 mm (H) fixed with both UV-C radiation and ultrasonic energy sources. It consists of four low pressure mercury UV-C lamps (18 x 4 W, CnLight Co. Ltd, Guangdong, China) with ballast and an immersible integral mounting flange type piezoelectric ultrasonic transducer (1 kW, 40 kHz, Branson Ultrasonic, Shanghai, China). It has a perforated stainless steel produce basket (9 in x 3.5 in x 3.5 in) in the middle and a steel based support platform with rollers and roller locks. The disinfecting equipment is operated at 1 kW ultrasonic energy at UV-C intensity of $640 \mu\text{W}/\text{cm}^2$ for two lamps (CT) or $900 \mu\text{W}/\text{cm}^2$ for 4 lamps (CF) configurations. For comparison of effectiveness from the two configurations, variations of treatment duration from 80 to 1200 s was created to achieve similar dosage levels ranging from $0.72\text{-}10.76 \text{ kJ}/\text{m}^2$ by multiplying each intensity with its respective treatment time.

C. Design of Experiment and Statistical Analysis

A completely randomized block design consisting 15 runs from 7 levels of UV-C radiation dosage from each UV-C intensity of 640 and $900 \mu\text{W}/\text{cm}^2$ and a control without UV-C and ultrasonic energy was conducted for a batch of tomato samples. A total of 135 fruits were used each for microbiological analysis and functional properties assessment. Each analysis was measured in triplicates and expressed as mean \pm standard deviation in tables and mean \pm standard error. The entire experimental design was repeated two times. Data analysis was made using Microsoft Excel 2016 (Microsoft Corporation, Redmond, USA). One-way analysis of variance (ANOVA) was performed to determine the effect of treatment, while differences among treatment means were separated using a post-hoc Tukey test at 95% confidence interval ($\alpha = 0.05$) for all pairwise multiple comparison with the aid of SigmaPlot statistical software version 12.0 (Systat software Inc., CA, USA). Mann-Whitney rank sum test (two sample t-test) was also performed to test significant difference between the treatment by the two UV-C lamp configurations.

D. Microbiological Study

Microbial loads of total aerobic bacteria and yeast and mold of treated and untreated tomato samples were evaluated by the method of Pinheiro *et al.*, 2015 [20] with modification. Using the quartering sampling technique, 25 g of sample and 225 mL of 0.1% sterile buffered peptone water (BPW) was homogenized in a stomacher

(BAGMAKER 400-P, Interscience, St Nom, France) and 10-fold serial dilutions obtained from blended homogenate tomato tissues using 9 mL of 0.1% sterile BPW. 0.1 mL of diluent was spread-plated onto nutrient agar (NA), incubated for 2-3 days at 37°C (Mettler INE-600, Mettler GmbH+Co, Schwabach, Germany) for total aerobic bacteria (TAB) count enumeration. Yeast and mold load was determined using potato dextrose agar (PDA) acidified with 10 % tartaric acid solution to a pH of 3.5 to inhibit bacterial growth [21] and incubated at 30°C (BD53, BINDER GmbH, Tuttlingen, Germany) for 3-5 days. At the end of incubation period, discrete colonies formed were enumerated using a digital colony counter (Galaxy 230, Rocker Scientific, New Taipei, Taiwan) using the standard plate count method [22] and expressed as colony forming unit per gram of fruit following equation below. The average value of three plates for each serially diluted homogenate was found and result recorded.

$$CFU/g = \text{number of colonies per ml/dilution factor}$$

E. Antioxidant Properties Assessment

Extracts were obtained from treated and untreated tomato samples following methods of Rigano *et al.*, 2014 and Del Giudice *et al.*, 2015 [3], [23] with modifications. 3 g of blended homogenate tomato tissue was transferred to a 50 ml falcon tube with the addition of 10 ml analytical grade methanol (90%), extracted in an ultrasound bath (Branson 5200 Ultrasonic Corp.) at 30°C for 60 min and subsequent centrifugation at $3500 \times g$ (Hettich, Universal 320, Andreas Hettich GmbH, Tuttlingen, Germany) for 10 min at 4°C . Filtration was achieved using a filter paper (Whatman No 1) to obtain hydrophilic extract stored at -5°C in glass bottles covered with foil for total phenolic compounds and hydrophilic antioxidant activity assay.

Total phenolic content of tomato samples was determined using the Folin-Ciocalteu method of Singleton *et al.*, 1999 [24] as modified by Del Giudice *et al.*, 2015 [23]. 62.5 μL of supernatant from extraction was mixed 62.5 μL of Folin-Ciocalteu's phenol reagent and 250 μL of sterile distilled water in a 5 mL Eppendorf tube and vortex. 625 μL of 7% sodium bicarbonate (Na_2CO_3) was subsequently added after 6 min followed by dilution with 500 μL of sterile distilled water and vortex. The absorbance of the mixture was read at 760 nm (Ultrospec 3100 Pro, Biochrom Ltd Cambridge, UK) in 1.5 ml plastic cuvette (1 cm optical path or width) against a blank of distilled water after incubation at room temperature in a dark room for 90 min. Samples were analysed in triplicates, and total phenolic contents of tomato samples was expressed as milligram Gallic acid equivalents (GAE) 100 g^{-1} fresh weight using standard Gallic acid curve (0.042 to $0.492 \text{ mg}/\text{ml}$; $y=3.7089x-0.0063$; $r^2=0.9965$).

Antioxidant activity was evaluated by the FRAP assay which directly measures the ability of antioxidants to reduce ferric tripyridyltriazine complex (Fe^{+3} -TPTZ) to ferrous complex (Fe^{+2} -TPTZ) at low pH. The method of

Giudice *et al.*, 2015 and Liu *et al.*, 2012 [23], [25] was used with slight modification. 150 μL of supernatant from extraction was mixed with 3 mL of FRAP reagent in a 5 mL Eppendorf tube, vortex and incubated for 30 min at room temperature in the dark. The absorbance of the product (ferrous tripyridyltriazine complex) was read at 593 nm (Ultrspec 3100 Pro. Biochrom Ltd Cambridge, UK) in 1.5 mL plastic cuvette (1 cm optical path) against a blank of distilled water. FRAP reagent used for the assay was freshly prepared for each day. 0.3 M acetate buffer having a pH of 3.6 was prepared from 16 mL acetic acid and 3.1 g $\text{C}_2\text{H}_3\text{NaO}_2 \cdot 3\text{H}_2\text{O}$ in 1000 mL sterile distilled water. 10 mM 2, 4, 6-tris (1-pyridyl)-5-triazine (TPTZ) was prepared by dissolving 0.0468 g TPTZ in 15 mL of 40 mM HCl at 50 $^\circ\text{C}$ in a water bath. 20 mM ferric solution ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was prepared by dissolving 0.0811 g in 15 mL sterile distilled water. Final working volume of FRAP was obtained by freshly mixing acetate buffer, TPTZ and ferric solution in a ratio of 10:1:1. 18 mL of sterile distilled water was added and maintained in a water bath at 37 $^\circ\text{C}$ for use. Results were expressed as mM Trolox Equivalent (TE) 100 g^{-1} of fresh weight from a standard Trolox curve (20 to 960 μM Trolox; $y=0.0017x-0.1395$; $r^2=0.9993$).

III. RESULTS AND DISCUSSIONS

A. Effect of Disinfection on Microbial Growth

Untreated tomato presented high levels of contamination with aerobic bacteria (4.66 ± 0.25 Log CFU g^{-1} FW) on nutrient agar, which have also been observed in previous studies [26], [27]. Reduction of total aerobic bacteria population increased with increasing dosage applications for both treatment configurations as observed in Fig. 1. This led to a maximum reduction of 2.03 and 2.33 logs respectively for 2 and 4 lamps configurations at dosage level of 6.46 kJ/m^2 . ANOVA results indicates significant effect ($p < 0.001$) of treatment dosage for each lamp configuration.

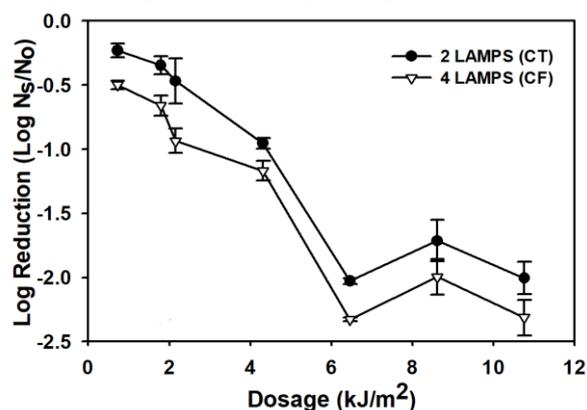


Figure 1. Effect of simultaneous UV-C radiation and ultrasonic energy Treatment on total aerobic bacteria reduction of tomato. Results are mean \pm standard error from three measurements

Analysis at low dosage from 0.72 - 2.15 kJ/m^2 and high dosage level from 4.31 - 10.77 kJ/m^2 separately also gave significant difference ($p < 0.05$) effect of log reduction of 0.23 - 0.94 and 1.17 - 2.33 logs respectively. Reference [27]

using single UV-C treatment observed that higher treatment dosages induced reasonable log reductions on initial aerobic mesophilic bacterial population, whereas lower dosages evoked little or no effect. Reference [20] using single ultrasound suggested that any treatment that lowers the level of microbial load development can be considered as an effective hurdle technology. As such, the simultaneous use of UV-C radiation and ultrasonic energy in this study is anticipated to be effective in disinfecting fresh tomatoes even at lower dosage levels.

For the study of yeast and mold population using tomatoes at the turning stage, only trace amount appeared on agar plates including the untreated samples. It was concluded that yeast and mold population on tomato could be dependent upon harvesting practices which starts from the field [28]. The investigation was required to be repeated using more ripened fruits, *i.e.* between 30 to 60% of surface aggregate showing pink or red colour to signal yeast and mold contamination.

Table I shows that the initial yeast and mold count for untreated samples stood at $3.36 \text{ log CFUg}^{-1}$. Previous studies have reported initial yeast and mold count of as low as $1.6 \text{ log CFUg}^{-1}$ and as much as $4.69 \text{ log CFUg}^{-1}$ on tomato [12], [20], [27], [29], [30]. The ripening stage of tomato sample could be responsible for the difference in yeast and mold population density as yeast and mold contamination is associated with ripeness. Yeast and mold survivors were not detected with the higher dosage treatment from 4.31 - 10.76 kJ/m^2 for both lamp configurations. Initial counts were reduced by 0.14 - 0.75 log by the lower dosage treatment from 0.72 - 2.15 kJ/m^2 significantly ($p < 0.05$). While Cote *et al.*, 2013 [31] observed no difference in the population of yeast and mold after exposure to 4 kJ/m^2 UV-C radiation, Mukhopadhyay *et al.*, 2014 [27] reported log reduction of 0.17 - 2.01 immediately after treatment with 0.6 - 6.0 kJ/m^2 UV-C radiation.

In general, the results of this simultaneous UV-C and ultrasonic energy treatment showed significance up to 2.33 log reductions for bacterial load and for yeast and mold, to the point of no survivors. Compared to other studies, single treatment gave 0.03 - 1.5 log reduction [20], [27], [29], [32], and combination of UV-C radiation and sanitizer formulation [12] gave 2.23 log reductions while combination of ultrasonic energy with slightly acidified electrolytic water [29] gave 1.80 log reductions. Ruptured cell envelopes of microorganism due to mechanical effects of ultrasonic cavitation ensured access facilitation of UV-C radiation to sensitive microorganisms' DNA. However, results from current study still fall short when compared with 3.4 log reductions in yeast and mold, and 4.4 in aerobic mesophiles [32] when ultrasonic was combined with peracetic acid. Reference [33] also obtained a 3 log reduction in human adenovirus (hAdVs) on tomato from the use of sequential combination of UV-C radiation and ultrasonic energy.

B. Effect of Disinfection on Total Phenolic Content

The overall concentration of total phenols (Fig. 2) was significantly higher ($p < 0.001$) in treated tomatoes where 2 and 4 lamps configuration at highest treatment dosage

gave values of 18.90 and 20.92 mg GAE 100g⁻¹ FW, respectively.

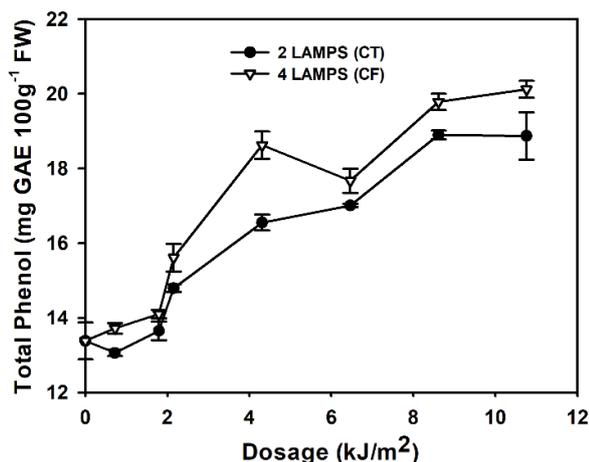


Figure 2. Changes in total phenolic content of tomato (mg GAE 100g⁻¹ FW) subjected to simultaneous UV-C and ultrasonic energy treatment. results are mean \pm standard error of three measurements

Combined UV-C radiation and ultrasonic energy treatment resulted in overall increase of 41.26-50.37% of total phenols from 13.38 mg GAE 100g⁻¹ FW for the 2 and 4 lamps configuration, respectively. This increase may be linked to the scavenging free radicals' antioxidant properties and excessive lignification as a response to phytotoxic effect of high dose UV-C radiation [34] and extreme conditions of pressure and temperature arising from ultrasonic cavitation implosion [35]. Reference [36] also pointed out that stress factors, especially such induced by UV-C and ultrasonic energy can lead to increased total phenol contents due to stimulated polyphenol production. Significant increases and higher phenolic content have been observed with UV-C radiation at dosage range of 1-8 kJ/m² [18], [25], [34], [37]-[39], 20 kJ/m² UV-B radiation [39], ultrasound [20], edible coating [5] and gamma radiation applications [40], [41].

In contrast, Pinheiro *et al.*, 2015 [42] reported that total phenol content of tomato treated with 0.32-4.83 kJ/m² UV-C radiation was not significantly different to control fruits. An indication from this study is that there could be

possibility that combined DNA-damaging effect of UV-C radiation with the strong ultrasonic mechanical cavitation energy induces accumulation of UV-C light which absorbs phenolic compounds and activates phenolic biosynthesis pathway, thus enhances the total phenols in tomatoes. Further statistical analysis using paired comparison between data from the 2 and 4 lamps shows non-significance ($p > 0.05$) between them even though the 4 lamps had higher phenolic content value than the 2 lamps.

C. Effect of Disinfection on Antioxidant Activity

Fig. 3 shows that samples had an initial hydrophilic antioxidant activity of 70.31 mMTE 100g⁻¹ FW. Values of the same quantum have been reported in literature, *i.e.* 0.75 mM Trolox kg⁻¹ FW [37], 78.72 μ M TEAC 100g⁻¹ FW [18], 599.40 μ M Trolox 100g⁻¹ [30], 420 μ M Trolox 100g⁻¹ [43], 187.43 μ mol TE 100g⁻¹ FW [23]. Simultaneous treatment has caused an increase in antioxidant activity for all dosage applications, and to the highest values of 95.67 and 89.65 mMTE 100g⁻¹ FW at 8.61 kJ/m². Statistical analysis showed no significant difference ($p > 0.05$) for both lamp configurations.

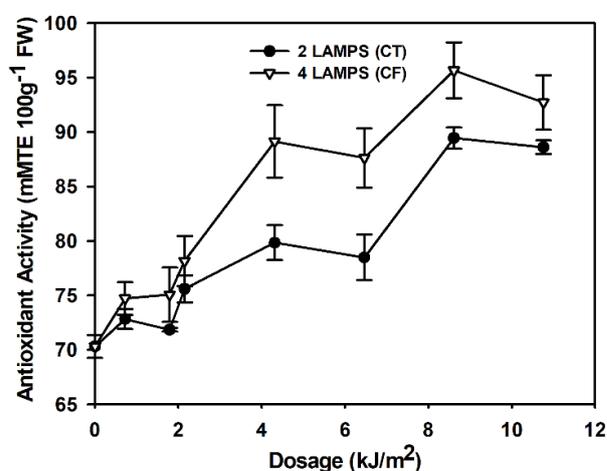


Figure 3. Changes in antioxidant activity of tomato (mMTE 100g⁻¹ FW) subjected to simultaneous UV-C and ultrasonic energy treatment. results are mean \pm standard error of three measurements

TABLE I. CHANGES IN MEAN YEAST AND MOLD POPULATION (LOG CFUG-1 FW) OF TOMATO SUBJECTED TO SIMULTANEOUS UV-C AND ULTRASONIC ENERGY

Control	Treatment Dosage (kJ/m ²)						
	0.72	1.79	2.15	4.31	6.46	8.62	10.76
Two Lamps Configuration (CT)							
3.36 \pm 0.24	3.01 \pm 0.16	3.22 \pm 0.53	NDS	NDS	NDS	NDS	NDS
Four Lamps Configuration (CF)							
3.36 \pm 0.24	2.90 \pm 0.07	3.05 \pm 0.21	2.61 \pm 0.02	NDS	NDS	NDS	NDS

NDS: no detectable survivors

This positive effect of treatment on antioxidant activity from hydrophilic extract agrees with earlier reported studies utilizing UV-C radiation [25], [30], [37], but contradicts with reports by Jagadeesh *et al.*, 2011 [18] and Liu *et al.*, 2011 [40] that hormetic UV-C and UV-B radiation dose had no significant effect on tomato hydrophilic antioxidant activity. Since there is a positive

correlation between antioxidant activity and total phenolic content ($r = 0.89$ for 2 lamps and 0.93 for 4 lamps), the increase in antioxidant activity could be attributed to the increase in total phenolic contents [36], [41], [44]. There is also non-significant difference ($p > 0.05$) difference between both lamp configurations.

IV. CONCLUSION

Simultaneous UV-C and ultrasonic energy disinfection had significantly ($p < 0.001$) inhibited aerobic bacteria and yeast and mold growth on tomatoes. In addition, it has also stimulated the accumulation of tomato phytochemicals of total phenolic content and antioxidant activity from its hydrophilic extract. The variation of intensity of UV-C radiation from the two and four lamp configurations however did not give any significant effects. The treatment level measured in dosage as a result of treatment duration was the most important factor in giving significant effects of disinfecting tomatoes and also increasing its functional properties of total phenolic content and antioxidant activity.

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