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Efficacy of Ultraviolet Irradiation on *shiga toxin Escherichia coli* Inactivation of Pineapple-Mango Juice Blend

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ABSTRACT

This study aims to evaluate the potential of ultraviolet irradiation treatment on shiga toxin E. coli O157:H7 in pineapple-mango juice blend as alternative to thermal pasteurization. Ultraviolet irradiation denotes as one of the non-thermal alternative technology for microbial inactivation in fruit juice processing. Tropical fruit well known with its unique flavor with pineapple and mango stands among the most popular tropical fruit. Pathogenic microorganism of E. coli O157:H7 in fruit juice able to cause fatality if consume. Pineapple and mango mixed together at ratio 70% pineapple and 30% mango. The result obtain indicates the efficiency of ultraviolet irradiation was comparable to thermal pasteurization as the shiga toxin E. coli O157:H7 reach 6 log reduction after treatment at ultraviolet dosage of 8.38mJ/cm². Ultraviolet irradiation proved to be promising in juice safety.

KEYWORDS

Ultraviolet irradiation, pineapple, mango, shiga toxin E. coli O157:H7

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INTRODUCTION

Fruit juice gaining well attention due to its beneficial effect, rich in vitamins and minerals content (Teja et al., 2017). Aneja et al. (2014) define juice as unfermented but fermentable for fresh consumption through extraction process of fully ripe fruits. Juice obtained from single fruit known as single fruit juice, while combination of two or more different types of fruit blends together called mixed fruit juice (Jorge, 2006) or also known as juice blends (Kamarul Zaman et al. 2016). Through blending of different juices, the nutritional attributes of juice can be improved (Jan and Masih, 2012).

Pineapple scientifically named as *Ananas comosus* L planted extensively in tropic and sub-tropic region (Shamsudin et al., 2007). Local name for pineapple of Josapine variety is "*Nanas madu*" due to its sweet taste. Pineapple unique flavor made it one of the popular tropical fruits consumed worldwide and utilized into juice and canned pineapple. Laorko et al. (2013) added that pineapple juice high in antioxidant and phenolic content which benefit consumer health. Pineapple juice either processed as single strength juice or mixed with other fruit juice producing new flavor (Carvalho et al., 2008). Josapine variety of pineapple is well known in Malaysia due to its early fruits times (Shamsudin et al., 2007) made it abundantly available.

Mango (Mangifera indica L.) known as queen of tropical fruit with distinctive taste is believed to be originated from Southeast Asia (Santhirasegaram et al., 2013). Mango fruits widely planted almost in 85 countries and rank fifth in production alongside bananas, citruses, grapes and apple (Santhirasegaram et al., 2015b). Chokanan mango cultivar is popular in Malaysia due to its off-season flowering even without chemical initiation (Santhirasegaram et al., 2015a). Speer et al. (2009) added that Chokanan mango has 3 harvesting period which are in May, June and August made it easy to be utilized into product such as juice, nectar and puree. However, Chokanan mango shelf life is short due to its fast ripening especially under ambient temperature (Ahmad Tarmizi et al., 1996). Thus, it is better for the fruit to be utilized into value added product.

Both pineapple and mango juice are in the acidic range of pH. Josapine pineapple and chokanan mango juice pH are 3.81 (Shamsudin et al., 2007) and 4.62 (Santhirasegaram et al., 2015a) respectively. Supposed that acidic juice will unlikely to be contaminated but previous outbreak on *Escherichia coli* O157:H7 shows otherwise with unpasteurized fruit juices (apple cider recoded as the most related outbreak) dominate the outbreak (Wareing and Davenport, 2016). Buchanan and Edelson (1996) state that *Escherichia coli* O157:H7 is acid-tolerant and able to survive at strong acid condition of 3.0 to 4.5. *Escherichia coli* O157:H7 contamination in food is mainly due to improper handling during processing (Basarn et al., 2004). Outbreak due to consumption of pineapple recorded in 1994 (Strawn et al., 2011) showing pineapple juice are expose to contamination. Hsin-Yi and Chou (2001) study on *Escherichia coli* O157:H7 in mango juice found that the pathogenic bacteria were able to survive up to 8 days at 7°C.

Thermal pasteurization is widely use in juice processing industry due to its efficiency in juice preservation. Mohd-Hanif et al. (2016) added that pathogenic bacteria were well disinfected in fruit juice after thermal pasteurization with increase in shelf life of juice. Prior studies on apple (Siguemoto et al., 2018), orange (Topalcengiz and Danyluk, 2017) shows thermal pasteurization able to inactive *Escherichia coli* O157:H7 to safety limit as regulated by FDA. However, thermal pasteurization causing quality degradation of treated juice due to high temperature applied (Laorko et al., 2013). Therefore, alternative non-thermal technology such as ultraviolet irradiation, pulsed electric field, high pressure and ultrasound were widely studied to overcome the adverse effect of thermal pasteurization.

Ultraviolet irradiation treatment at wavelength ranges from 200 to 280 nm known as UV-C were well acknowledge for its ability to kill microorganism (Teja et al., 2017) with 254 nm is the optimum wavelength (Santhirasegaram et al., 2015a). Caminiti et al. (2012) added that short wave UV-C able to be absorbed by the microorganism DNA, generating some modification to the chemical structure thus, lead to cell destruction. The efficiency of ultraviolet irradiation treatment towards microorganism is presented by the ultraviolet irradiation dosage. Different microorganism will react differently at different dosage induced during treatment (Koutchma, 2009). Ultraviolet irradiation treatment on apple and cranberry juice blend shows that treatment at same dosage ($5.3 J/cm^2$) result with greater *E. coli* reduction compare to yeast (Palgan et al., 2011). Although the use of ultraviolet irradiation for *Escherichia coli* O157:H7 inactivation proved promising reduction, up to date no study being made on pineapple-mango juice blends. Therefore, this study aims to analyze the effectiveness of ultraviolet irradiation on *shiga toxin Escherichia coli* O157:H7 inactivation compare to thermal pasteurization.

MATERIALS AND METHOD

Pineapple-mango juice preparation

Matured Josapine pineapple and Chokanan mango fruits were obtained from local retail in Selangor. Pineapple fruit was peeled, eye removed, washed and cut into smaller pieces. Mango fruit was washed, peeled, and cut into smaller pieces. Both pineapple and mango fruits were extracted individually using juice extractor (Power Juice, Smart ShopTM, US), filter using muslin cloth and centrifuged to obtained clear juice. Following Kamarul Zaman et al (2016) centrifugation was done at 9000 rpm for 15 minutes (Benchtop Centrifuge, Universal 320/320 R, Hettich Zentrifugen, Germany). Juice then blended together at blending ratio of 70% pineapple and 30% mango as Kamarul Zaman et al. (2016) stated that the selected ratio results with best ascorbic acid, pH, color properties and less turbid compare to other ratio (50%pineapple:50%mango and 30%pineapple:70%mango).

Shiga toxin Escherichia coli O157:H7 (STEC) inoculation

Due to the numerous foodborne outbreak of E. coli O157:H7 in fruit juice (Vojdani et al., 2008) STEC was chosen to be inoculated in pineapple and mango juice blends. STEC was obtained in Tryptic Soy Agar (TSA) from Bacteriological Food Safety Laboratory, Food Science and Technology Faculty, University Putra Malaysia. A loop of STEC bacteria were cultured in Tryptic Soy Broth (Bacto™, Becton, Dickinson and Company, USA) incubated for 24 hours at 37°C (the remaining culture in TSA refrigerated at 4°C for further used). Following procedure by Tosun and Gönül (2006), STEC cell pellet was obtained and diluted in 0.1% peptone water (10^{-1} to 10^{-5}) and plated on sorbitol McConkey agar (SMAC) (Difco™, Dickinson and Company, USA) and incubated at 37°C for 24 hours. STEC colonies observed will be pink in colour on the SMAC agar. Total plate count (TPC) and yeast and mould count (YMC) were also analysed in this study. TPC was done follows the procedure as describe by Maturin and Peeler (2001), while YMC follows AOAC 2007 methods.

Thermal and ultraviolet irradiation treatment

Ultraviolet irradiation treatment was done using ultraviolet pasteurizer (Malaysia patent (PI201203186), similar with prior studies by Mansor et al. (2014), Mansor et al. (2017), Mohd-Hanif et al. (2016a) and Mohd-Hanif et al. (2016b). The ultraviolet reactor consists of 6 low pressure mercury lamps (Philips, Malaysia) with 5 quartz lamps coiled with polyfluoroalcoxy (PFA) tube and one uncoiled lamp. Each lamp was 28 mm diameter and 915 mm length vertically arranged. In this study one coiled lamp and middle uncoiled lamp were used. The ultraviolet irradiation dosage follows Mansor et al (2014). Figure 1 shows the schematic diagram of process flow of ultraviolet pasteurizer used.

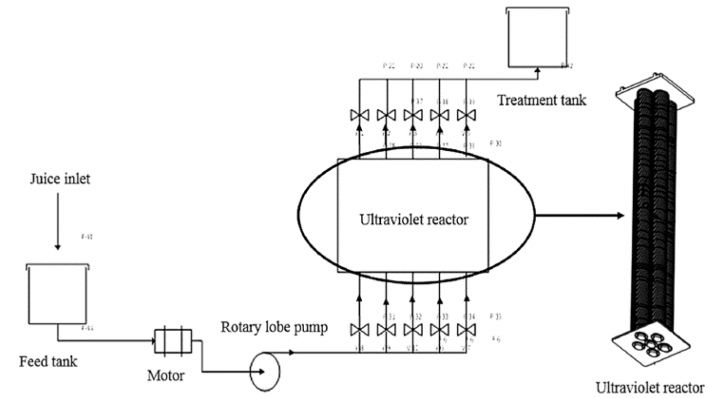

Figure 1: Schematic diagram of ultraviolet pasteurizer (Adapted from Mohd-Hanif et al., 2016a)

Comparison was made with thermal pasteurization. Thermal pasteurization of pineapple and mango juice blend was done at 90°C for 5 minutes. Pasteurization process was done using batch pasteurizer (P9000, Elecrem, France).

RESULTS AND DISCUSSIONS

UV-C dosage for microorganism inactivation in pineapple-mango juice blend

Ultraviolet irradiation treatment efficiency depends greatly on the applied dosage on the treated sample. Based on Table 1 pineapple-mango juice blends at flow rate of 0.0116 L/s result with higher UV-C dosage compare to juice flow at flow rate 0.0137 L/s despite the minimal different of the flow rate. This study consistence with previous research on pineapple (Mansor et al., 2014) and lime (Mohd-Hanif et al., 2016b) juice using the same ultraviolet pasteurizer in which at higher pump flow selected, the UV-C dose produce was lower. This was due to shorter time of interaction between juice flowing inside the ultraviolet pasteurizer with UV lamp at higher pumping capacity. Frequency of pump flow was measured in Hertz (Hz) which is cycle per second. 40 Hz indicate the juice flow at 40 cycles per second. According Mansor et al. (2014) high frequency cause lower RTD and relatively results with higher juice flow rate, hence lower UV-C dose.

The flow pattern inside the PFA coiled tubing ultraviolet pasteurizer was turbulence for both designated pump frequency. Fluid having Reynolds number of >4000 and <2000 are turbulent and laminar flow respectively. Mansor et al. (2014), study on pineapple juice treated at UV-C dose ranging from 10.10±0.7 – 13.75±1.5 mJ/cm^2 all having laminar flow pattern. Laminar flow pattern may cause inconsistency of UV-C dose distribution along the ultraviolet reactor (Koutchma et al., 2004). The result obtain in the current study deviate from the prior study as pineapple-mango juice blend exhibit turbulent flow pattern for both designated pump frequency. Turbulence flow more effective in microorganism disinfection as the targeted microorganism brought closer to the ultraviolet light source (Koutchma et al., 2007). Apple juice and apple cider recorded higher log reduction of E. coli O157:H7 in turbulent treatment compare to laminar flow treatment (Sauer and Moraru, 2009)

Table 1: Ultraviolet irradiation (UV-C) parameters

Pump flow (Hz)	Flow rate (L/s)	RTD (s)	UV-C dose (mJ/cm^2)	Reynolds no
40	0.012	8.65	8.38	5093
45	0.014	7.28	6.47	6053

RTD is residence time distribution

Comparing the microorganism inactivation in pineapple-mango juice blends, UV-C dose of 8.38mJ/cm^2 was more efficient with greater log reduction recorded. Although, the different in UV-C dose was minimal, due to the instable flow at higher frequency, microorganism was unable to be fully exposed to the light source results with lower inactivation rate. Referring to Figure 2, *shiga toxin Escherichia coli* O157:H7 (STEC O157:H7) was reduce to 7.4 log reduction which meet the FDA regulation of 5 log reduction of pertinent pathogen in fruit juice (FDA, 2016). Similarly, E. coli O157:H7 in apple cider was effectively inactive up to 6.65 log reduction after UV-C treatment at 14mJ/cm^2. The UV-C dosage in present study were lower compare to prior studies due to the processing steps in which pineapple and mango was first centrifuged to obtained clearer juice in order to reduce the limitation of ultraviolet irradiation treatment on turbid and opaque liquid. Keyser et al. supported that apple juice only require low dosage of ultraviolet treatment compare to orange juice to effectively inactive *E. coli* K12 as apple juice was clear than orange juice.

Usually, UV-C treatment were less effective in yeast and mould inactivation due to the DNA structure of yeast that exhibit higher resistance towards chemical changes and require higher dosage to be inactive (Bhat et al., 2011). Present study showed that YMC log reduction in 8.38mJ/cm^2 was the lowest compared to TPC and STEC but still more than 5 log reduction. While, at 6.47mJ/cm^2 UV-C dose YMC, TPC and STEC was under the permissible limit indicate inadequate dosage induce for the treatment. Charles et al. (2009) observed E. coli was effectively inactive while yeast and lactic acid was unable to be completely reduced after treatment. However, UV-C treatment at 0.032W/cm^2 results with no detection of microorganism of yeast/mould and mesophilic bacteria in apple juice (Juarez-Enriquez et al., 2016) indicate the dosage induce was enough to inactive the microorganism below the detection limit.

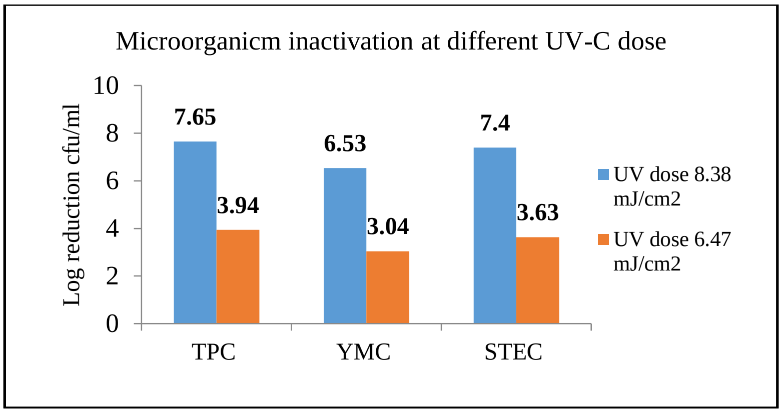

Figure 2: Microorganism inactivation at different ultraviolet irradiation (UV-C) dosage (TPC, YMC and STEC are total plate count, yeast and mould count and shiga toxin Escherichia coli O157:H7 respectively)

Thus, UV-C dose of 8.38mJ.cm^2 was chosen as the effective dosage for inactivation of STEC O157:H7 in pineapple-mango juice blends.

Effect of ultraviolet irradiation and thermal pasteurization on microbial inactivation

Thermal pasteurization was known to be widely used in juice processing industry as it proven to be effective to kill microorganism in fruit juice. Table 2 shows the initial microbial counts in pineapple-mango juice blends before and after treatment with thermal and ultraviolet irradiation treatment.

Table 2: Microbial counts at difference pasteurization treatment

Treatment	TPC (log cfu/ml)	YMC (log cfu/ml)	STEC (log cfu/ml)
Fresh juice	7.65	7.79	7.40
UV-C (8.38mJ.cm^2)	0	1.26	0
Thermal pasteurization (90°C, 5 min)	0	0	0

(TPC, YMC and STEC are total plate count, yeast and mould count and *shiga toxin Escherichia coli* O157:H7 respectively)

Thermal pasteurized pineapple-mango juice blend was safe to be consume as all microorganism available in the juice was inactive. From the results, UV-C treatment was effective in STEC inactivation, comparable to thermal pasteurization. Similarly, *E. coli* O157:H7 in apple cider (Basaran et al., 2004; Donahue et al., 2004: Sauer and Moraru, 2009), orange (Oteiza et al., 2010), clear apple (Alonzo, 2012 and Usaga et al., 2016), tamarind (Mohd-Hanif et al., 2016a) juice were effectively reduced to limit allowable as regulated by FDA (5 log reduction).

As discussed earlier, ultraviolet irradiation unable to completely reduce the counts in yeast microorganism but the counts were still low. Tran and Farid (2004) added that despite the effectiveness of ultraviolet treatment on microorganism inactivation, the sensitivity of treatment differ for each microorganism as microorganism develop different reaction towards the cell destruction by UV-C.

CONCLUSION

Ultraviolet irradiation treatment on pineapple-mango juice blend show promising results of microbial reduction at higher UV-C dose. The study could be improved through adjustment of pump frequency to enhance the fatality rate of microorganism especially yeast and mould that harder to be disinfected. Other parameter of juice turbidity and optical density may be explored in future study to enhance effectiveness of ultraviolet irradiation in juice processing. Ultraviolet irradiation treatment proved to be comparatively effective in inactivation of *shiga toxin Escherichia coli* O157:H7 in pineapple-mango juice blends.

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