

Effects of UVC irradiation and thermal treatment on the physico-chemical properties and microbial reduction of clear and turbid tamarind juice

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Abstract

Tamarind juice is commonly treated with thermal treatment to inactivate microorganisms. However, thermal treatment deteriorates the appearance and flavor of tamarind juice. In this study, the performance of UVC treatment on clear and turbid tamarind juice as an alternative to thermal treatment was investigated. Results showed that UVC treatment was able to reduce *E. coli* O157:H7 by 7-log reduction but unable to completely remove the total bacterial population as observed in thermally treated clear and turbid tamarind juices. The pH of treated tamarind juices was comparable to each other. Nonetheless, UVC treatment caused significant changes on the flavor of the juice as it significantly reduced the total soluble solids of tamarind juices and decreased the titratable acidity of turbid tamarind juice. However, UVC treatments retained the appearance i.e. turbidity and color of tamarind juices better than thermal treatment.

Keywords

Ultraviolet
Thermal treatment
Tamarind juice
Physico-chemical property
Microbial reduction

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Introduction

Tamarind (*Tamarindus indica* L.) juice has a sweet and tasty taste that makes it popular in cooking, beverages and as an ingredient in traditional health remedies. Nonetheless, the current method of preparing the juice is by soaking the pulp in hot or cold water and squeezing out the extracts by hands which is inconvenient and is prone to contamination (Lee *et al.*, 2009). Previously, *Escherichia coli* O157:H7 has been reported to cause foodborne illnesses of unpasteurized apple juice and cider (Sastry *et al.*, 2000). This implies that direct consumption of unpasteurized fruit juice without any treatment may be unsafe.

Thermal pasteurization is the most common method used to treat tamarind (*Tamarindus indica* L.) juice. Thermal pasteurization is effective in inactivating pathogens and bacteria hence, extending the shelf life of a juice. Nevertheless, thermal pasteurization results in adverse changes in flavor and appearance of tamarind juice (Lee *et al.*, 2009). Similarly, several reports had demonstrated that thermal treatments affect the total soluble solids (Tandon *et al.*, 2003; George *et al.*, 2015; Kaya *et al.*, 2015), turbidity (Igual *et al.*, 2013) and color (Choi and Nielsen, 2005) of fruit juices.

Ultraviolet-C (UVC) irradiation technique has been considered as an alternative treatment for pasteurizing fruit juices. This treatment minimizes the adverse effects of thermal pasteurization, low in cost and has high-energy efficiency compared to other treatments (Koutchma, 2008). According to Koutchma (2008), the U.S. Food and Drug Administration (FDA) and US Department of Agriculture (USDA) have concluded that the use of UV irradiation in food processing is safe and approved its usage in pasteurizing fruit juices. However, the treated juice is subjected to a requirement of at least 5-log reduction in the pertinent pathogen.

Regardless, the application of UVC in pasteurizing fruit juices is still limited. This is due to the low penetration capacity of UV in the presence of particles or soluble solids (Krishnamurthy, 2006). Consequently, fruit juices with different turbidity, total soluble solids, pH level and viscosity require different approaches in treating them with UV irradiation. Furthermore, many studies on UV pasteurization were conducted on fruit juices that are popular in Western countries such as apple, orange and grape juices. There is little information on its effectiveness on tropical juice. To date, there is no known research reported on the performance of UV irradiation in treating tamarind juice. Therefore, the objective of

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this study was to compare the effectiveness of UVC and thermal treatment in inactivating microbial load while retaining the physico-chemical properties of clear and turbid tamarind juice.

Materials and Methods

Juice preparation and *E. coli* O157:H7 inoculation

Commercial tamarind pulp was purchased from a local market in Selangor, Malaysia. Tamarind juice of 5% concentration was prepared by soaking tamarind pulp in deionized water for 15-30 minutes. The juice was filtered into a clear juice using a muslin cloth. Another set of tamarind juice was prepared but filtered using 500 μ m industrial sieve which results in a more turbid appearance. All juices were stored in a chiller (TD-1600, Protech, USA) at 4-8°C. Prior to experiment, *E. coli* O157:H7 was inoculated into tamarind juice to achieve an initial target concentration of 10⁷-10⁸ CFU/ml. The juice was treated within one hour after inoculation.

UVC treatment

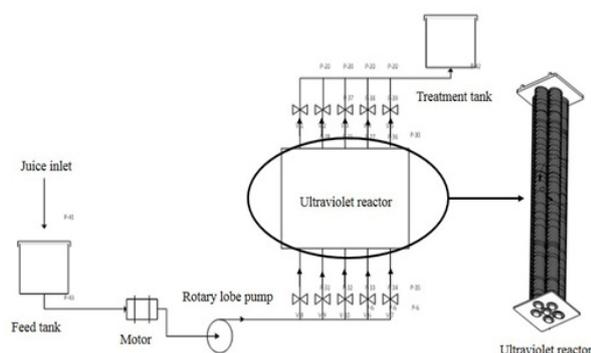


Figure 1. Flow process of UVC treatment (Mansor, 2015)

A UVC reactor (Malaysian Patent: PI201203186) was used as shown in Figure 1 (Mansor *et al.*, 2014; Mansor *et al.*, 2015). The reactor consists of five ultraviolet-C (UVC) lamps (HF-Performer; Philips, Malaysia), which emit UVC light at 254nm. The lamps were arranged in a circular manner vertically to each other. Each UVC lamp was enclosed with quartz glass sleeve and coiled with polyfluoroalcoxy (PFA) tubing (3.18mm OD and 1.65mm ID). Juice was pumped from the feed tank using a rotary lobe pump (S2; Xylem Water Solutions Ltd., Hoddesdon, UK) and flowed into five inlets of PFA tubing. The flow rate of the juice was manipulated by adjusting the pump's frequency. The flow rate will determine the exposure time of a treatment hence, the UV dose used. In this study, the pump was set at 35Hz as the flow rate was stable at this frequency. UV doses used were calculated based on Equation (1) (Chia *et al.*,

2012). The irradiance intensity was measured from UV radiometer sensors (UVX Radiometer; UVP Inc., Upland, CA, USA). The resulting UVC dosages calculated for clear and turbid tamarind juice at 35Hz were 35.50 and 35.22mJ/cm², respectively.

$$UV\ Dose\ (mJ/cm^2) = \frac{Irradiance\ Intensity\ (mW/cm^2)}{Exposure\ Time\ (s)} \quad (1)$$

Thermal treatment

A batch pasteurizer (P9000, Elecrem, France) was used. Tamarind juice was poured into a container and placed in a water bath inside the pasteurizer. The pasteurizer was covered completely with a lid. The juice was heated to 80°C for 5 minutes. The pasteurized juice was poured into sterilised glass bottles and sealed with sterilised caps.

Microbiological analysis

Untreated and treated juices were serially diluted and spread plated on triplicates of Trypticase Soy Agar (TSA) (Merck, Germany) and MacConkey Agar (MAC) (Merck, Germany). TSA was used to enumerate total viable bacteria while MAC was used to selectively enumerate *E. coli*. The colonies were counted by using a standard plate count method to quantify the colony forming units per ml (CFU/ml).

Determination of pH

The juice pH was determined by using a pH meter (PH25+, Crison, Barcelona, Spain) at room temperature (24 \pm 2°C).

Determination of titratable acidity (TA)

10 ml of juice sample was added to 40ml of distilled water. A few drops of phenolphthalein were added to juice sample as a color indicator. The juice was then titrated with standardized 0.1N sodium hydroxide (NaOH) until it reached the end point where a definite pink color was observed. The volume of titrant was used to calculate titratable acidity (%), expressed as percentage of tartaric acid as per equation (2):

$$Titratable\ acidity\ (\%) = \frac{volume\ of\ NaOH\ used\ (ml) \times 0.075 \times 0.1N\ NaOH}{sample\ volume\ (ml)} \times 100\% \quad (2)$$

Determination of total soluble solids (TSS)

Total soluble solids of juice was measured using a digital refractometer (AR-2008, Kruss, Hamburg, Germany). The refractometer was blanked using distilled water. A few drops of juice was placed onto a reading cell. The total soluble solids value was expressed as °Brix value.

Determination of turbidity

The turbidity of tamarind juice was measured using a turbidimeter (TN-100; Eutech Instruments Pte., Ltd., Singapore). Prior to analysis, the turbidimeter was calibrated with standard solutions of 0.02, 20.0, 100 and 800NTU. Juice needs to be diluted if the turbidity exceeds 1000NTU. The true turbidity of the juice can be calculated from the equation below (Canitez, 2002).

$$\begin{aligned} \text{True Turbidity (NTU)} \\ = \frac{\text{Turbidity(NTU)} \times (\text{volume of water} + \text{volume of juice})(\text{ml})}{\text{volume of juice}(\text{ml})} \end{aligned} \quad (3)$$

Determination of color

Color was determined by using Spectrophotometer UltrascanPro (D65, Hunter Lab, USA). The color was evaluated in terms of lightness (L^*), redness/greenness (a^*) and yellowness/blueness (b^*). These values were used to calculate hue angle (h°), chroma and overall differences of color (ΔE) using the equations below (MCLellan *et al.*, 1995; Caminiti *et al.*, 2012; Chia *et al.*, 2012).

$$h^\circ = \arctan(b^*/a^*) \quad (4)$$

$$\text{Chroma} = \sqrt[3]{(a^{*2} + b^{*2})} \quad (5)$$

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})} \quad (6)$$

Statistical analysis

Data collected were analysed by using SPSS 21.0 software. Data were represented as a mean value \pm standard deviation ($n=3$) and analysed using analysis of variance (one-way ANOVA) with Tukey's test at a significance level of $p < 0.05$.

Results and Discussion

Microbiological analysis

E. coli O157:H7 was inoculated in the juice to mimic contamination of juice by pertinent pathogen. It can be seen from Figure 2 that there was no colony count observed on MAC plates after UVC treatment in both juices which suggest that *E. coli* colony was successfully inactivated to below detection level. UVC treatments were able to reduce *E. coli* colony by 7-log reductions. On the other hand, the remaining colony on TSA plates are possibly surviving background microflora or any surviving *E. coli* that could not be retrieved on MAC plates. The inactivation of total viable bacteria in clear and turbid

tamarind juice as enumerated on TSA plates were significant. UVC treatment successfully reduced the total viable bacteria by 5.21 and 5.12 log CFU/ml in clear and turbid tamarind juice, respectively. Based on these results, the microbial reductions by UVC treatment exceeds the requirement of at least 5-log reduction as stated by the FDA. These findings are in line with Mansor (2015) who stated that the reduction of *Salmonella* Typhimurium in pineapple juice using the same UVC reactor was more than 5-log reduction. As depicted in Figure 2, it is clear that thermal treatments resulted in complete eliminations of microbes in clear and turbid tamarind juices. Similarly, Parish (1998), Rivas *et al.* (2006), and Santhirasegaram *et al.* (2015) reported that thermal treatment reduced microbes in fruit juices to below detection limit. From microbiological analyses, it can be concluded that UVC irradiation is not as efficient as thermal treatment. Nonetheless, UVC treatment improves the product safety, particularly with regard to *E. coli* O157:H7.

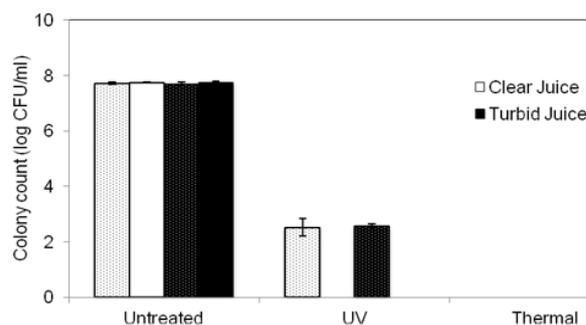


Figure 2. Inactivation of background microflora combined with *E. coli* O157:H7 in tamarind juice. Dotted columns indicate colony on TSA. Solid columns indicate colony on MAC.

Effects on pH and titratable acidity

Table 1 and Table 2 listed the physico-chemical properties of UVC- and thermally treated of clear and turbid tamarind juices, respectively. pH and acidity are important parameters in evaluating food quality. According to the FDA (2008), pH and/or acidity are used to determine processing requirements. Acid foods may not require sanitization process because most bacteria cannot survive in acidic condition. The pH of untreated, UVC-treated and thermally treated clear juice was 3.05, 3.11 and 3.03, respectively. The change in pH of UVC-irradiated juice was statistically significant ($p < 0.05$) but had no practical significance. Similarly, the pH of turbid tamarind juice showed minimal changes ($p < 0.05$) after UVC and thermal treatments as depicted in Table 1. These results are in line with a study by Santhirasegaram *et al.* (2013) on UV and thermal pasteurization of Chokanan mango where changes in pH between untreated (4.62) and pasteurized juice samples (4.59-

4.60) were not significant. In another study, Aguilar-Rosas *et al.* (2007) stated that pulsed electric field and thermal treatment showed significant statistical difference, however, the small discrepancies (3.8-3.9) had no practical significance and may be due to experimental error.

Titrateable acidity (%) contributes to the juice characteristic flavor. The titrateable acidity of untreated, UVC- and thermally-treated clear juices were 0.41, 0.42 and 0.43%, respectively which show insignificant changes ($p>0.05$). Nonetheless, the titrateable acidity of UVC-irradiated (0.40%) was significantly lower ($p<0.05$) than untreated (0.47%) and thermally treated turbid juices (0.49%). This could be due to the photochemical reactions of carboxylic acids such as tartaric acid by UVC light (Wang *et al.*, 2014). Similarly, Bhat *et al.* (2011) reported that the titrateable acidity of starfruit juice had decreased from 6.73% to 6.24% after UV treatment ($p<0.05$).

Table 1 Physico-chemical properties of UVC- and thermally treated clear tamarind juice

Properties	Untreated	UVC	Thermal
pH	3.05±0.01 ^a	3.11±0.01 ^b	3.03±0.01 ^a
TSS (°Bx)	3.5±0.1 ^a	2.8±0.2 ^b	3.8±0.1 ^c
TA (%)	0.41±0.01 ^a	0.42±0.01 ^a	0.43±0.01 ^a
Turbidity	144±6 ^a	143±9 ^a	168±1 ^b
L*	26.31±0.13 ^a	25.34±0.41 ^a	25.23±1.20 ^a
Hue angle (°)	71±4 ^a	79±1 ^a	75±1 ^a
Chroma	1.44±0.08 ^a	1.45±0.10 ^a	1.31±0.23 ^a
ΔE	-	1.0±0.4	0.5±0.1

*Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$).

Table 2 Physico-chemical properties of UVC-and thermally treated turbid tamarind juice

Properties	Untreated	UVC	Thermal
pH	3.12±0.01 ^{ab}	3.16±0.01 ^b	3.09±0.02 ^a
TSS (°Bx)	3.4±0.1 ^a	2.7±0.1 ^b	3.8±0.0 ^b
TA (%)	0.47±0.03 ^a	0.40±0.03 ^b	0.49±0.01 ^a
Turbidity	913±6 ^a	872±43 ^a	862±184 ^a
L*	25.36±1.50 ^a	26.88±0.89 ^a	26.90±0.62 ^a
Hue angle (°)	75±5 ^a	76±2 ^a	75±2 ^a
Chroma	2.22±0.92 ^a	2.13±0.43 ^a	2.02±0.74 ^a
ΔE	-	1.1±0.8	1.6±0.6

*Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$).

Effects on total soluble solids

The total soluble solids of UVC-treated clear tamarind juice (2.8°Brix) were lower than untreated (3.5°Brix) and thermally treated juice (3.8°Brix) ($p<0.05$). Likewise, the total soluble solids of turbid tamarind juice (3.4°Brix) decreased significantly

after UVC treatments (2.7°Brix) but increased after thermal treatment (3.8°Brix). It is clear that UVC treatment reduced the total soluble solids content in tamarind juice. The decrease in total soluble solids is possibly due to the photolysis of fructose by UVC light (Elsinghorst & Tikekar, 2014). Kaya *et al.* (2015) found that the total soluble solids of lemon-melon juice decreased from 8.72°Brix to 8.51°Brix after UV treatment. On the other hand, thermal treatment increased the total soluble solid of juice which is probably due to evaporation of water during heating (Tandon *et al.*, 2003) or as a result of hydrolysis of polysaccharides (Bal, 2012). However, these changes were not significant ($p>0.05$) similar to the findings of Pala and Toklucu (2011) and Santhirasegaram *et al.* (2015).

Effects on turbidity

Turbidity of juice is important especially when the appearance of a product matters. The turbidity of UVC-irradiated clear tamarind juice (143NTU) closely resembled the turbidity of fresh clear tamarind juice (144NTU). In contrast, the turbidity of clear tamarind juice was increased (168NTU) after thermal treatment ($p<0.05$). Yen and Lin (1998), Rivas *et al.* (2006) and Kaya *et al.* (2015) found that the turbidity of guava, blended orange and carrot and lemon-melon blend juices showed an increase in turbidity after thermal treatment. According to Rivas *et al.* (2006), thermal treatment causes precipitation due to degradation of pectin.

Unlike clear tamarind juice, there was no significant effect of treatments ($p>0.05$) on the turbidity of turbid clear juice as depicted in Table 2. Prior studies by Shamsudin *et al.* (2014) and Mansor (2015) reported a decrease in turbidity of pineapple juice after UVC treatment which could be due to inactivation of yeast and mould in the juice (Canitez, 2002). Meanwhile, Zhang *et al.* 2016 stated that the lower turbidity value of thermally treated carrot juice is possibly related to the lower viscosity value of juice after treatment. The change in turbidity in clear tamarind juice was more prominent compared to in turbid juice since it has a more transparent appearance.

Effects on color

Another crucial parameter evaluated was color. Food color is usually associated with the perception of juice quality and consumers' preference (Cinquanta *et al.*, 2010). L* value range from 0 (black) to 100 (white) measures the lightness of the juice. From Table 1 and Table 2, there were no effects of treatments on the lightness of clear (25.23-26.31)

and turbid (25.36-26.90) tamarind juices ($p>0.05$). Likewise, hue angle, h° which defines the type of color and chroma, which indicates the intensity of the color, did not change significantly in clear and turbid tamarind juices after UVC and thermal treatments ($p>0.05$). Nonetheless, the chroma of turbid juices (2.02-2.22) were slightly higher than that of clear tamarind juices (1.31-1.45). These differences are due to the amount of suspended solids and pigments in turbid tamarind juices which results in higher $+a^*$ and $+b^*$ values. Similar results were also obtained by Shamsudin *et al.* (2014) where UVC treatment did not change the lightness, hue angle and chroma of pineapple juice.

The overall color change (ΔE) was calculated to classify whether the color change is noticeable. The color change is “slightly noticeable” when ΔE is between 0.5-1.5 and “noticeable” if ΔE is between 1.5-3.0 (Caminiti *et al.*, 2012). In this study, the color change of UVC-irradiated and thermally treated clear tamarind juices were 1.0 and 0.5, respectively. These results suggest that the overall color change of both juices were “slightly noticeable”. On the other hand, the ΔE of thermally treated turbid juice was 1.6 which is slightly higher than UVC-irradiated turbid tamarind juice (1.1). The color change of thermally treated turbid juice falls in “noticeable” range whereas the UVC-irradiated turbid tamarind juice had a “slightly noticeable” change. These findings are in agreement with a study by Kaya *et al.* (2015) which reported that the overall color change in UV-treated juice is slightly lower (0.6) than in thermally-treated juice (0.9). Likewise, it has been reported that thermal treatment on pomegranate juice deteriorated the color of the juice due to degradation of anthocyanin pigments (Pala and Toklucu, 2011).

Conclusion

The performance of UVC irradiation on the inactivation of *E. coli* O157:H7 was comparable to thermal treatment. However, UVC treatment was not able to completely inactivate the total bacterial population as observed in thermally treated tamarind juice. Both treatments showed comparable properties in terms of the pH. However, the total soluble solids of clear and turbid tamarind juices were greatly reduced by UVC treatments. Likewise, UVC treatments significantly reduced the titratable acidity of turbid tamarind juice. On the other hand, thermal treatment increased the turbidity of clear tamarind juice and changed the color of turbid tamarind juice. These results suggest that UVC treatment may not be suitable in pasteurizing tamarind juice due

to its lower microbial inactivation efficiency and compensable physico-chemical properties to thermal pasteurization. However, a combined treatment i.e. UV-thermal could be useful to overcome the limitations of both treatments.

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