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UVC Dosage Effects on the Physico-Chemical Properties of Lime (*Citrus aurantifolia*) Juice

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Abstract Lime juice is in high demand due to a sour taste. Commercial thermal pasteurization extends juice shelf-life; however, fruit juice subjected to thermal pasteurization tends to change color and lose vitamin content. Lime juice was irradiated with ultraviolet-C (UVC) at dosages of 22.76, 30.19, and 44.24 mJ/cm^2 to investigate effects on the physicochemical properties of lime juice. pH values of lime juice did not change while total soluble solids, turbidity, titratable acidity, sweetness, and color values of lime juice did change after UV treatments. Changes in quality index indicators were prominent at the highest UV dosage of 44.24 mJ/cm^2. A low UVC dosage was effective for treatment of lime juice with minimal changes in juice properties.

Keywords: ultraviolet, dosage, Dean Vortex, lime juice, physicochemical property

Introduction

Citrus is one of the most important fruit crops in the world due to its high value in international trade. Lime (*Citrus aurantifolia*), a typical citrus fruit, is a green and round fruit of 3-6 cm in diameter that is usually harvested green for commercial use, turning yellow when ripe. Limes are traditionally grown in Asian countries, such as India and Thailand, and are an essential ingredient in many cuisines due to a unique sour taste. In contrast with other types of citrus fruits, such as oranges, tangerines, and grapefruits, lime has a higher percentage of citric acid than of sugar content (1). Limes are abundant in vitamin C and are often used for flavor and aroma enhancement in foods and beverages and as medicines.

Lime juice is usually produced via manual squeezing of fresh limes. However, this practice is unhygienic and not suitable for long term use. Furthermore, in some countries such as Thailand, the price of limes varies by season (2). The price is low during the rainy season but may increase up to 20 times during the summer. Due to these factors, there is a need for commercially pasteurized lime juice products in order to serve consumer demands and to maintain price stability. Currently, there are a number of commercially pasteurized lime juice products sold in the market. However, the majority of lime juice products in the market are not 100% natural due to addition of artificial acids and flavors during processing (2).

Thermal pasteurization and ultra high temperature (UHT) sterilization are processing methods that are commonly used for treatment of lime juice in order to increase safety and product shelf-life. Unfortunately, excessive thermal treatment can cause deterioration in quality attributes of the final product. Previous study of lime juice has demonstrated that total soluble solids, pH, viscosity, color, flavor, appearance, and overall liking values of lime juice significantly deteriorated after thermal pasteurization (2). Application of heat inactivates most enzymes, oxidizes some vitamins, and degrades food components that contribute to the color and taste of juice.

Non-thermal technologies have been introduced as alternatives to overcome the drawbacks of thermal pasteurization. Ultraviolet-C (UVC) irradiation, a non-thermal processing technique, has potential in treatment of fruit juices. However, the efficiency of UVC treatment is highly influenced by amounts of suspended solids, soluble solids, and UV-sensitive compounds, and by the initial microbial load and flow conditions (3). Hence, Dean Vortex technology has been introduced to increase the efficiency of UVC treatment. In this system, juice flows in a helically coiled tube around a UV lamp. Because of the curved tube, juice flows under the influence of centrifugal forces that create secondary eddy flow vortices called Dean Vortices (4). Formation of secondary eddy flow enhances mixing and the microbial inactivation efficiency of UV treatments.

To date, no information is available on the effects of UVC irradiation in lime juice. Therefore, this study investigated the performance of UVC irradiation with Dean Vortex technology as an alternative to thermal pasteurization. Effects of UVC dosage on the physicochemical properties of lime juice were studied to determine the optimum dosage for treatment.

Materials and Methods

Lime juice preparation Limes (*Citrus aurantifolia*) were purchased from a local market in Selangor, Malaysia. After washing and cleaning, the skin was peeled off to obtain the fruit flesh. Juice was then obtained using a juice extractor (HR2826; Philips, Amsterdam, the Netherlands). Prepared juice was stored in a chiller (ProtechTD-1600; Tech-Lab Scientific Sdn. Bhd, Seri Kembangan, Malaysia) at 4°C prior to use.

UVC treatment UV reactor design based on Dean Vortex technology was used (Fig. 1) (5). Low-pressure mercury UV lamps were used as UVC sources due to electromagnetic emission at 254 nm. Poly-fluoroalcoxy (PFA) (3.18 mm OD and 1.65 mm ID) tubes were coiled around five low-pressure mercury UVC lamps (HF-Performer; Philips) to create secondary eddy Dean flow for enhancement of mixing and the UVC microbial killing efficiency (6).

Lime juice flowed into five PFA tubing inlets from a tank using a rotary lobe pump (S2; Xylem Water Solutions Ltd., Hoddesdon, UK). Juice flow rates were adjusted based on pumping frequency where a low frequency resulted in a low flow rate and a long UVC exposure time. Pump frequencies were set to 25, 30, and 35 Hz to achieve stable lime juice flow rates. The exposure time or residence time distribution was calculated based on division of the tube volume by the flow rate. The intensity of irradiation, which was directly recorded using a UV radiometer (UVX Radiometer; UVP Inc., Upland, CA, USA) was used for calculation of UV dosage with respect to the pump frequency (Eq. 1) (7). Calculated UV dosages are listed in Table 1.

Table 1. Calculated UVC dosages

Frequency (Hz)	Flowrate (m^3/s)	Residence time distribution(s)	UV dosage (mJ/cm^2)
35	5.21×10^{-6}	48.03	22.76
30	3.92×10^{-6}	63.84	30.19
25	2.85×10^{-6}	87.96	44.24

$$\text{UV Dose } (mJ/cm^2) = \text{Irradiance intensity } (mW/cm^2) \times \text{Residence time Distribution (s)} \quad (1)$$

Physicochemical and color analysis All parameters were measured before and after UVC treatment. pH was measured using a pH meter (PH25+; Crison Instruments, Barcelona, Spain). The total soluble solids value (TSS) was directly measured using a refractometer (AR-2008; Kruss GmbH, Hamburg, Germany). TSS values were reported as °Brix. A turbidimeter (TN-100; Eutech Instruments Pte., Ltd., Singapore) was used for measurement of lime juice turbidity in Nephelos Turbidity Units (NTU). Due to the high turbidity of lime juice, 0.5 mL of lime juice was diluted with 9.5 mL of distilled water once prior to each measurement. The true turbidity was calculated as (Eq. 2):

$$\text{True turbidity (NTU)} = \frac{\text{turbidity} \times (\text{volume of distilled water} + \text{volume of juice sample (mL)})}{\text{volume of juice sample (mL)}} \quad (2)$$

Titratable acidity (TA) was measured as a percentage where 10 mL of lime juice was diluted with 40 mL of distilled water with addition of a few drops of phenolphthalein indicator. The mixture was titrated using a 0.1 N NaOH solution. The indicator changed from colorless to pink at the end point. The initial and final volumes of NaOH were recorded in mL. Titratable acidity was calculated based on the following equation (Eq. 3) where 0.064 is the milliequivalent factor of citrus acid:

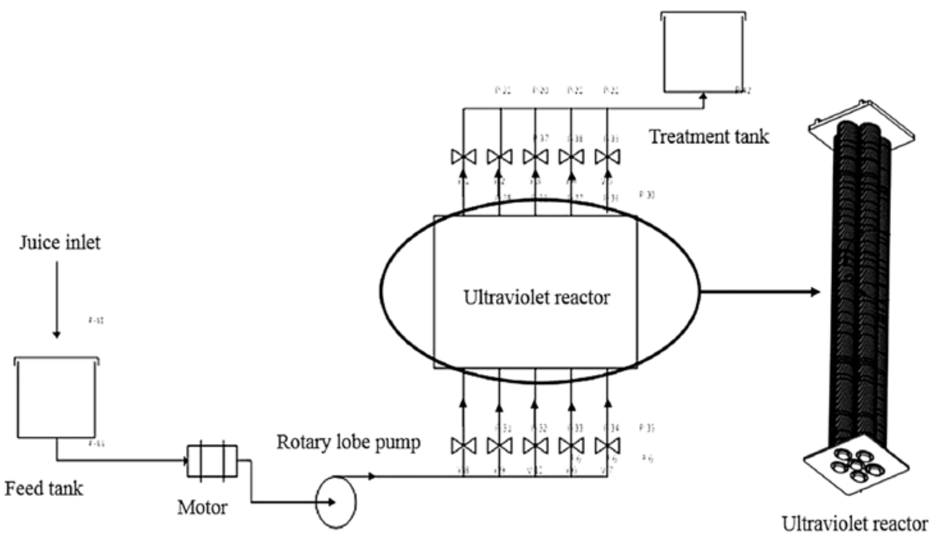

Fig. 1. UV reactor design (5).

T.A. (%)

$$=\frac{\text{volume of NaOH used (mL)}\times\text{molarity of NaOH}\times 0.064}{\text{volume of sample (mL)}}\times 100\% \quad (3)$$

Color was measured using a spectrophotometer UltrascanPro (D65; HunterLab, Reston, VA, USA) to obtain L*, a*, and b* values. Hue angle (Eq. 4), chroma (Eq. 5), and color difference (ΔE) (Eq. 6) were calculated to express changes in color.

$$\text{Hue angle } (^\circ)=\tan^{-1}(b^*/a^*) \quad (4)$$

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

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

Statistical analysis Experiments were carried out in triplicate. For each replicate, triplicate measurements were obtained for each analysis and averaged. Statistical analysis was performed using a one-way analysis of variance (ANOVA) with SPSS software at a significance level of $p\leq 0.05$. Results were reported as a mean± standard deviation.

Results and Discussion

Physicochemical properties of lime juice are shown in Table 2 where fresh, untreated lime juice is shown as 0 mJ/cm^2.

pH pH is important for determination of the processing conditions of a product. pH values of lime juice were in a range between 2.34-2.35. Lime is highly acidic. Hence, not many bacteria could survive. However, a previous report on the presence of *Escherichia coli* 0157:H7 in unpasteurized apple cider (8) showed that pasteurization is required to ensure that acid-resistant bacteria are eliminated. In this study, there were no significant (p>0.05) changes in pH values under different UV dosages from 22.76-44.24 mJ/cm^2 (Table 2), compared with the control. It is apparent that UVC irradiation did not alter the pH of lime juice. Since there is no or minimum amount of heat involved in UV pasteurization, changes in pH values were expected to be negligible, in agreement with previous reports on pineapple, lemon-melon, mango, grape, and orange juices where changes in pH were not significant (9-13).

Total soluble solids (TSS) TSS is important as an indicator of the amount of sugar or acid that should be added to a juice. Sugars are the main soluble solids of fruit juice. Other soluble solids include organic and amino acids, soluble pectins, and vitamins. The TSS value decreased significantly (p<0.05) from 7.9 to 7.4, 6.0, and 5.1°Brix at 22.76, 30.19, and 44.24 mJ/cm^2, respectively. TSS values decreased with an increasing UV dosage. Some soluble solids contain conjugated structural bonds that are susceptible to UVC attack, leading to degradation or alteration of soluble solid components hence the decreased in TSS value. Application of high UV dosages resulted in greater reduction in TSS value, so a low UV dosage was preferred.

Titratable acidity (TA) TA is another crucial parameter that contributes to juice flavor. Likewise, the TA value of UVC-treated lime juice decreased significantly (p<0.05) from 5.48 to 3.00% with an increasing UV dosage from 22.76 to 44.24 mJ/cm^2. TA changes were significant (p<0.05) and more prominent at 30.19 and 44.24 mJ/cm^2 where the TA values recorded were 3.86 and 3.00%, respectively. Thus, high UV dosages exacerbated reduction in TA values. Bhat *et al.* (14) demonstrated that UVC irradiation of starfruit juice resulted in reduction of TA values from 6.73 to 6.24%. Reduction in TA values could be due to degradation of alpha hydroxyl acid in lime juice (15). UVC irradiation is absorbed by double bonds in the alpha hydroxyl acid structure, opening the bonds and allowing reaction with neighboring molecules, leading to degradation.

Turbidity Turbidity is an indication of juice cloudiness or haziness. Lime juice turbidity values decreased significantly (p<0.05) with increasing UV dosages (Table 2). The turbidity value of fresh lime juice was 12,567 NTU. Turbidity values of lime juice at 22.76 and 30.19 mJ/cm^2 significantly (p<0.05) decreased from 12,567 to 11,060 and 8,670 NTU, respectively. The turbidity value was significantly (p<0.05) reduced by 50% after lime juice was irradiated at 44.24 mJ/cm^2. Reduction in turbidity could be due to inactivation of yeast and mold in fruit juice (9), which can contribute to juice haziness and turbidity. Therefore, application of high UV dosages inactivated greater amounts of yeast and mould and, hence, decreased turbidity values of lime juice. Cruz-cansino *et al.* (16) also reported that sonication caused fragmentation of colloidal pectin molecules, which resulted in less sedimentation.

Sweetness Sweetness was measured as a ratio of TSS to TA values. The TSS/TA ratio of fresh lime juice and juices treated at 22.76, 30.19, and 44.24 mJ/cm^2 were 1.42, 1.48, 1.54, and 1.70, respectively. Sweetness significantly (p<0.05) increased as the UV dosage increased,

Table 2. Physico-chemical properties of lime juice

UV dosage (mJ/cm^2)	pH	TSS (°Brix)	Turbidity (NTU)	TA (%)	TSS/TA
0	2.34±0.01^{a}[1)]	7.9±0.10^{a}	12,567±375^{a}	5.48±0.10^{a}	1.42±0.02^{a}
22.76	2.35±0.01^{a}	7.4±0.10^{b}	11,060±424^{b}	5.06±0.25^{a}	1.48±0.09^{b}
30.19	2.35±0.01^{a}	6.0±0.10^{c}	8,670±42^{c}	3.86±0.22^{b}	1.54±0.07^{c}
44.24	2.34±0.01^{a}	5.1±0.10^{d}	6,070±31^{d}	3.00±0.07^{c}	1.70±0.15^{d}

[1)]Values with the same letter in the same column indicate no significant difference (p>0.05).

Table 3. Color parameters of fresh and UVC-treated lime juices

UV Dose (mJ/cm^2)	L*	a*	b*	Hue angle	Chroma	ΔE
0	61.47±0.05$^{a1)}$	-3.12±0.02^a	8.54±0.03^a	110.04±0.18^a	9.09±0.02^a	0
22.76	60.94±0.23^a	-2.98±0.06^{a,b}	6.85±0.06^b	113.58±0.50^b	7.47±0.02^b	1.78
30.19	58.49±0.59^b	-2.93±0.02^b	4.53±0.06^c	122.92±0.31^c	5.40±0.06^c	5.02
44.24	55.28±0.08^c	-2.55±0.04^c	1.64±0.02^d	147.28±0.62^d	3.03±0.07^d	9.30

[1)]Values with the same letter in the same column indicate no significant difference (p>0.05).

due to reduction in TA values that was more prominent than reduction in TSS values after UV treatment. For instance, at the highest UV dosage, reduction in TA values was 45% whereas the decrease in TSS values was 35%. Since lime juice is mainly used for a sour taste, an increase in sweetness is not favorable.

Lime juice color Product appearance plays an important role in consumer acceptability (17). Color is correlated with the appearance of a product. In this study, color values were expressed as L*, a*, b*, hue angle, chroma, and ΔE values. Lightness (L*) is defined as brightness of a color. The lightness value ranges from 0 (black) to 100 (white). The lightness of fresh lime juice was 61.47 whereas UV-treated lime juices exhibited significantly (p<0.05) lower lightness values ranging between 55.28 and 60.94 at 22.76 to 44.24 mJ/cm^2, indicating that treated lime juices turned darker.

The a* value represents greenness (–a*) and redness (+a*) of a color. Fresh lime juice exhibited a value of −3.12, which indicated more greenness, compared with lemon (−1.233) and apple (−1.738) juices (18). After UVC treatment, a* values significantly (p<0.05) decreased to −2.98, −2.93, and −2.55 at 22.76, 30.19, and 44.24 mJ/cm^2, respectively. Similar trends were observed for b* values, which indicate the blueness (–b*) and yellowness (+b*) of a color. Fresh lime juice exhibited a b* value of 8.54, which is leaning towards yellow. However, the yellowness values of lime juices significantly (p<0.05) decreased to 6.85, 4.53, and 1.64 at 22.76, 30.19, and 44.24 mJ/cm^2, respectively. According to Ibarz *et al.* (19), TSS is highly correlated with a* and b* values where the a* and b* values of lemon juice were found increases with increasing TSS. In this study, UVC treatment caused reduction in TSS of lime juice hence, its a* and b* values.

The hue angle (°) represents a specific red, blue, yellow, or green color, or any combination of colors. The hue angle of fresh lime juice was 110.04° whereas juices irradiated at 22.76, 30.19, and 44.24 mJ/cm^2 exhibited values of 113.58, 122.92, and 147.28°, respectively (Table 3). Thus, hue angles significantly (p<0.05) increased with an increasing dosage, compared with the control. Hue angle values were in the yellow region, similar to unaided observations. Chroma indicates the intensity of a color. A low chroma value indicates a grey shade or tone. The intensity of lime juice colors changed significantly (p<0.05) from 9.09 to 7.47, 5.40, and 3.03 at 22.76, 30.19, and 44.24 mJ/cm^2, respectively. While hue angle and chroma represent color and intensity, ΔE indicates whether a change in color is noticeable. A ΔE value above 3 indicates a visible change. The color of lime juice changed significantly (p<0.05) after irradiation with UVC , compared with controls, (Table 3). Slightly noticeable changes of ΔE=1.78 were recorded for the lowest UVC radiation dosage of 22.76 mJ/cm^2. However, the highest dosage of 44.24 mJ/cm^2 resulted in a calculated ΔE value of 9.3, which indicated a greatly visible change.

Maillard reactions between reducing sugars, such as glucose and fructose, and amino acids in lime juice probably resulted in browning, causing color changes (2). Furthermore, color degradation could also be caused by isomerization of carotenoids and oxidation reactions from interactions with free radicals generated during irradiation (18). Results presented herein were in agreement with other studies of mango, starfruit, and pomegranate juices for which UVC irradiation changed the juiced color (11,14,20).

In conclusion, juice flavor, based on TSS and TA values, and appearance, based on turbidity and color values of lime juice, changed after UVC treatment. Changes in physicochemical properties were minimal at a low UVC dosage of 22.76 mJ/cm^2. Therefore, use of a high UVC dosage for treatment of lime juice should be avoided.

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Disclosure The authors declare no conflict of interest.

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