

# Inactivation of *Saccharomyces cerevisiae* and Polyphenoloxidase in Mango Nectar Treated with UV Light

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## ABSTRACT

Fresh mango nectar was processed by UV light at five flow rates (0.073 to 0.451 liter/min) and five UV light doses (75 to 450 kJ/m<sup>2</sup>) to evaluate total microbial load, *Saccharomyces cerevisiae* survival, and polyphenoloxidase activity. UV systems containing an inner mercury lamp (254 nm) each with intensity of 25 mW/cm<sup>2</sup> were used as germicidal sources. In addition, mango nectar was treated for 15 min at 0.073 and 0.451 liter/min, stored at 3°C, and evaluated periodically for total microbial count, yeast count, color, and polyphenoloxidase activity. The first-order kinetics modeling found that  $D_{UV}$ -values in mango nectar ranged from 27.9 to 10.9 min ( $R^2 > 0.950$ ) and 26.0 to 11.8 min ( $R^2 > 0.962$ ) for total microbial count and yeast count, respectively. The maximum log reduction (CFU per milliliter) was 2.71 and 2.94 for total microbial count and yeast count, respectively, after 30 min of UV treatment at 0.451 liter/min.  $D_{UV}$ -values ranging from 156 to 204 min were observed for polyphenoloxidase activity. The remaining polyphenoloxidase activity after 30 min of UV treatment at 0.451 liter/min was  $19 \pm 4\%$ . Initial microbial load and yeast in stored mango nectar were reduced in the range 2.86 to 3.41 and 1.82 to 1.97 log (CFU/ml) cycles, respectively. No substantial microbial growth was observed prior to 20 days of storage. Averages of  $1,055 \pm 32$ ,  $803 \pm 32$ , and  $710 \pm 37$  enzyme activity units were observed in mango nectar UV processed at 0, 0.073, and 0.451 liter/min, respectively, during the entire storage period. However, mango nectar treated at 0.073 and 0.451 liter/min maintained a yellow and yellow-orange color, respectively, after 26 days of storage.

Today, UV light (UV-C; 254 nm) treatment is a disinfection method used to inhibit or inactivate foodborne and/or pathogen microorganisms in foods (12). UV-C light is a nonthermal technology, along with pulsed electric fields and high hydrostatic pressure, among other novel nonthermal technologies (4).

Generated by UV mercury lamps, UV-C light between 200 and 300 nm can have a germicidal effect on bacteria, viruses, protozoa, molds, and yeasts (21). The UV light effect on microorganisms will depend on the type of microorganism, species, strain, stage of culture, growth media (8), density of microorganisms, and type and composition of food. The DNA in the nucleus absorbs UV light, which can be harmful to the cell, leading to cell inactivation and finally to cell death (16, 30). The UV energy absorbed in the cell may interfere with DNA replication and transcription. Pyrimidine dimers, some pyrimidine adducts, and pyrimidine hydrates are called “photoproducts,” which can promote cross-linking with proteins (28).

UV light treatment is commonly used for sterilization of water (1, 15), air (28), and surfaces such as materials nonresistant to heat sterilization (3) and other types of surfaces (28). For food surfaces, UV light has been used on fresh fruits, vegetables, and roots to reduce the microbial load, or as a “hormetic effect,” to improve the resistance against detrimental microorganisms when stimulating the formation of phytoalexins (31). On the other hand, UV irradiation is also a commercial cold process used in com-

bination with H<sub>2</sub>O<sub>2</sub> for sterilization of packaging materials, but not for food products (23).

For liquid food products, pasteurization is the standard method used to obtain microbiologically safe products; however, characteristics such as change in color and aroma and loss of vitamins may occur with increased temperature. The use of UV light on liquid foods has not been studied as a disinfection method (10) because UV light has a very low penetration effect on turbid and colored liquids (29). Consequently, today it is more commonly used to reduce microbial loads in water (6). UV-C light can penetrate 4 m in air, about 300 mm in clear water, only 1 mm in fruit juices, and no more than 0.1 mm in milk (17). Therefore, it is advisable to use a turbulent flow so that UV light reaches all parts of the liquid treated in the tubes, since soluble solids, suspended matter, particle size, and color can all block light from reaching the microorganisms (6, 28). The use of thin films of liquid foods is also recommended to enhance the efficiency of UV light penetration and to guarantee lethality against bacteria (27).

Microbial reduction in apple juice flowing through pipes has been studied. The microbial survival curves follow first-order kinetics when representing survival microbial load as a function of processing time (at constant intensity) or fluence (dose) (13). Thus,  $D_{UV}$ -values can be obtained to describe the decimal reduction time at constant flow rate similar to  $D$ -values obtained from thermal processing at constant temperature (7). However, shoulders or plateaus on the curve at low UV-C doses may be found for some types of microorganisms (28).

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Polyphenoloxidase is one of the enzymes that can produce discoloration changes in fruit products. The enzymatic browning can be observed in fruits and vegetables after they are cut and exposed to air and its substrates. Polyphenoloxidase can react with monophenols and diphenols to undergo oxidation and generate orthoquinones, which polymerize to form brown pigments (melanins) (19, 25). However, no information has been found about the use of UV light processing to inactivate enzymes in liquid fruit products such as mango nectar.

The objective of this study was to evaluate the UV light effect on *Saccharomyces cerevisiae* and polyphenoloxidase inactivation in fresh and stored mango nectar. Selected doses of UV-C light and flow rates for mango nectar were used for microbial reduction. UV-C light treated mango nectar stored at 3°C was also evaluated for shelf life.

## MATERIALS AND METHODS

**Mango nectar.** Mature mangos (*Mangifera indica*) purchased at a local supermarket in Pullman, Wash., were washed, peeled, and deseeded. The puree was obtained using a domestic blender and stored in a glass beaker surrounded with ice water (1 to 2°C) before making the nectar. Forty percent (wt/wt) of mango puree was mixed with water, in addition to sugar and citric acid, to obtain a nectar with 13°Brix and ratio of sugar/acidity of 55/1 (wt/wt). Mango nectar was prepared three times to be UV light processed.

**Microbial growth.** Two milliliters of frozen *S. cerevisiae* (ATCC 10274) was obtained from our stock (1 ml of microorganisms grown in early stationary phase, plus 1 ml of sterile glycerol [20 ml glycerol/100 ml water] stored at -21°C). The unfrozen *S. cerevisiae* was then added to 100 ml of Sabouraud broth (Difco, Becton Dickinson, Sparks, Md.) for growth on a rotary platform shaker at 30°C and 225 rpm until reaching the early stationary phase or 27 h maximum time.

**Microbial count.** Serial dilutions were made before pour plating in standard methods agar (BBL, Becton Dickinson, Sparks, Md.) and dichloran-rose bengal-chloranphenicol agar (Difco, Becton Dickinson), for total microbial count or *S. cerevisiae*, respectively. Total microbial count was taken after storing for 24 to 48 h at 35°C, and *S. cerevisiae* was counted after 5 days in storage at 22°C (room temperature) (2).

**PPO assay.** Polyphenoloxidase (PPO) activity was evaluated at 30°C using an 8452A diode array spectrophotometer (Hewlett-Packard, Palo Alto, Calif.) at 420 nm. Five milliliters of nectar was mixed with 5 ml of McIlvaine buffer (pH 6.6) prepared with citric acid (Sigma-Aldrich, St. Louis, Mo.) and dibasic sodium phosphate (Sigma-Aldrich) into polystyrene sterile centrifugal tubes. The enzyme extract was obtained by centrifuging the mixture for 40 min at 1,630 × *g* (4°C) and then filtering it through Whatman paper no. 1. The reaction mixture consisted of 1 ml of buffer, 0.5 ml of catechol (0.175 M), and 0.25 ml of enzyme extract. The linear portion obtained by plotting the reaction time versus absorbance was used to compute the enzyme activity units (EAU). One unit of PPO activity was defined as 0.001 Δ*A*<sub>420</sub> min<sup>-1</sup> ml<sup>-1</sup> (14, 24). All extracts were analyzed in triplicate.

**Equipment.** Two Infinity liquid disinfection systems (Atlantic UV, Co., Hauppauge, N.Y.) made of stainless steel were coupled in a serial arrangement to make one UV-disinfection unit (Fig. 1). Each disinfection system holds 0.25 liter (*V*) of liquid in the annular space between the wall of stainless steel and the sleeve (made of quartz). The UV lamps (power = 25 W; diameter = 15

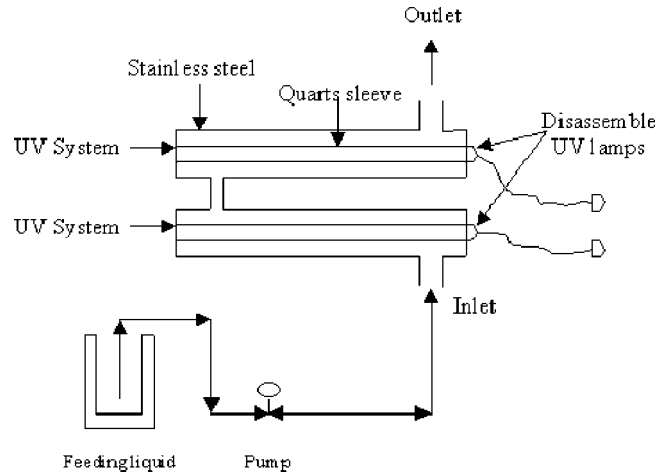


FIGURE 1. Two coupled UV-disinfection systems to treat mango nectar.

mm; length = 556 mm) placed inside each disinfection system were warmed up for a minimum of 30 min before starting to circulate the liquid. The UV light is transmitted at a length of 46.5 cm to the disinfection area (262 cm<sup>2</sup>). The intensity (*I* = 25 mW/cm<sup>2</sup>) was measured on the surface of the lamp using a digital radiometer with a silicon photoelectric sensor (Cole Palmer Instrument Co., Vernon Hills, Ill.). Digital thermometers (Cole Palmer) were placed at the entrance and exit of the UV-disinfection unit to measure temperature.

**UV processing.** Eight hundred milliliters of mango nectar was measured into a glass beaker surrounded with ice water (1 to 2°C). The inoculated (1 ml culture per 100 ml nectar) mango nectar was pumped and recirculated into the UV-disinfection unit using a Masterflex I/P peristaltic pump (Cole Palmer) at flow rates (*q*) of 0.073, 0.165, 0.255, 0.349, and 0.451 liter/min. The processing time for each flow rate was 5, 10, 15, 20, 25, and 30 min, corresponding to dosages of 75, 150, 225, 300, 375, and 450 kJ/m<sup>2</sup>, respectively. The residence time (*θ*) was obtained dividing *V* (0.25 liter) by each flow rate. Also, noninoculated mango nectar was UV treated at 0.073 and 0.451 liter/min for 15 min and then stored in culture glass tubes at 3°C for 30 days. Stored UV processed mango nectar was analyzed periodically for 1 month.

**Modeling.** Microbial reduction or enzyme inactivation was analyzed using a first-order kinetic model:

$$\ln\left(\frac{A_t}{A_0}\right) = -kIt = -kF \quad (1)$$

where *A<sub>t</sub>* is the residual microbial load (*N<sub>t</sub>* = CFU per milliliter) or residual enzyme activity (*E<sub>t</sub>* in EAU) at a given time, *A<sub>0</sub>* is the initial microbial load (*N<sub>0</sub>* = CFU per milliliter) or initial enzyme activity (*E<sub>0</sub>* in EAU), *k* is the inactivation constant rate (square meter per kilojoule), *I* is the intensity (watts per square meter), *F*(*It*) is the dosage or fluence (kilojoules per square meter), and *t* is the exposure time (seconds).

**Decimal reduction dose.** This is the dose required to inactivate 90% of microorganisms or enzyme activity at a given flow rate, and it is calculated as follows:

$$D_{UV} = -\frac{2.303}{k} \quad (2)$$

where *D<sub>UV</sub>* (kilojoules per square meter) is the decimal reduction dose and *k* is the inactivation constant rate (square meters per kilojoule).

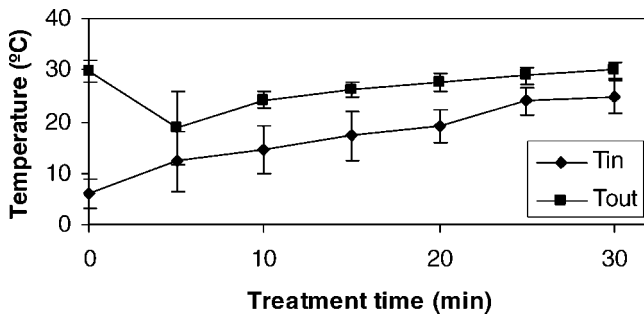


FIGURE 2. Temperature increase in UV light-processed mango nectar.

**$Z_{UV}$ -value.**  $Z_{UV}$  is the change in flow rate required to obtain a 10-fold change in the inactivation dose, and it is calculated as follows:

$$\text{Log } D_{UV} = -mq \quad (3)$$

where  $m$  is the slope (minutes per liter),  $q$  is flow rate (liters per minute), and  $Z_{UV}$  (liters per minute) is computed from the negative inverse of the slope (12, 13).

**Color.** Light to dark ( $L^*$ ) (100 to 0), red (+) to green (-) ( $a^*$ ), and yellow (+) to blue (-) ( $b^*$ ) color parameters were determined using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in the reflection mode. Ten milliliters of mango nectar was measured into a small white round plastic container (diameter = 40 mm; height = 10 mm). A white ceramic plate was used for standardizing the instrument ( $L^* = 93.4$ ,  $a^* = -0.67$ ,  $b^* = 0.78$ ). A Hunterlab standard white plate, no. W823, was used as background for measuring color parameters ( $L^* = 93.10$ ,  $a^* = -0.65$ ,  $b^* = -0.85$ ). The color difference ( $\Delta E^*$ ) was calculated as follows (18):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4)$$

**Statistical analysis.** Linear regression and standard deviation were performed using a Microsoft Excel program (Microsoft Inc., Redmond, Wash.). The analysis of variance (ANOVA) and least significant difference were calculated with the SAS program (26). A  $P$  of 0.05 was used for making decisions about significant differences. Microbial load (total count or yeasts), PPO units, or color parameters ( $L^*$ ,  $a^*$ ,  $b^*$ , or  $\Delta E^*$ ) were the variables used for the ANOVA procedure as a function of flow rates and times. All analyses were performed in triplicate.

## RESULTS AND DISCUSSION

**Mango products.** The total soluble solids for puree and nectar were  $13.3 \pm 0.6$  and  $13.0 \pm 0.1$ , respectively.

TABLE 1. Residence time, number of passes, and dose per pass of UV light-processed mango nectar at different flow rates

Flow rate (liter/min)	Residence time (min)	NP <sup>a</sup>		Dose/pass (mJ/cm <sup>2</sup> )
		(5 min)	(30 min)	
0.073	3.425	1.5	8.8	5,135
0.165	1.515	3.3	19.8	2,265
0.255	0.980	5.1	30.6	1,470
0.349	0.716	7.0	42.0	1,080
0.451	0.554	9.0	54.1	825

<sup>a</sup> Number of passes throughout the UV system = NP -  $\nu\theta$ .

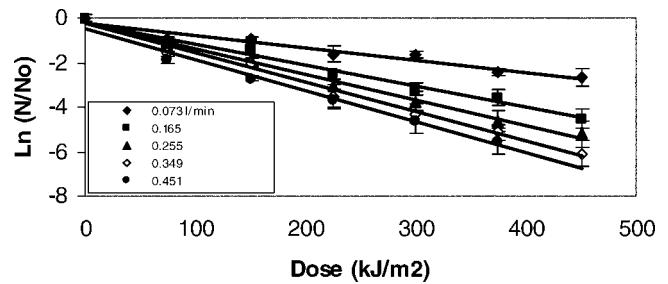


FIGURE 3. First-order kinetic modeling of microbial total count in UV light-processed mango nectar at different flow rates.

Acidity of puree ( $0.7 \pm 0.07\%$  of citric acid) was the only characteristic that decreased, since the addition of water and sugar diluted the mango puree when making the nectar ( $0.3 \pm 0.03\%$  of citric acid). However, citric acid was also added to maintain the ratio of 55/1 (wt/wt) of sugar/acid. Mango puree ( $\text{pH} = 3.9 \pm 0.2$ ) and nectar ( $\text{pH} = 3.8 \pm 0.2$ ) are considered high-acid fruit products in which growth of *Clostridium botulinum* can be inhibited due to their low pH (4.6 to 3.7) (7).

**Flow characteristics.** Figure 2 depicts the increase in temperature and standard deviation of UV-treated mango nectar for all experimental flow rates and treatment times. Even though mango nectar was recirculated through the UV-disinfection unit several times, according to processing time and flow rate (Table 1), the maximum temperature reached by the nectar before entering ( $T_{in}$ ) and leaving ( $T_{out}$ ) the UV-disinfection unit was  $24.9 \pm 3.2^\circ\text{C}$  and  $29.9 \pm 1.6^\circ\text{C}$ , respectively, after 30 min of treatment. Therefore, this UV light process can be considered nonthermal since temperatures between 60 and  $100^\circ\text{C}$ , even for a few seconds or minutes, are commonly used in thermal processing (4). On the other hand, an increase in flow rate increased the number of passes ( $\text{NP} = t/\theta$ ) through the UV-disinfection unit (Table 1). Without taking into account the processing time (5 to 30 min), the doses per pass (Table 1) decreased as flow rate increased because residence time decreased as flow rate increased. Therefore, less energy is required to inactivate more microorganisms at higher flow rates (Figs. 3 and 4). Consequently, more UV-treated liquid fruit product is obtained with less energy. However, there are some limits to flow rate when inactivating both total microbial count and yeast count, which will be discussed below.

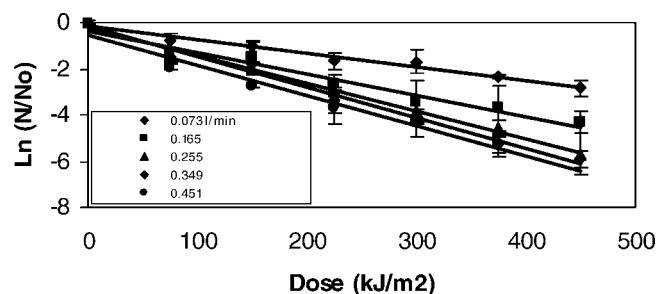


FIGURE 4. First-order kinetic modeling of yeasts in UV light-processed mango nectar at different flow rates.

TABLE 2. Log reductions and coefficient of correlation for total microbial count and yeast count in UV light-processed mango nectar at different flow rates

Flow (liter/min) <sup>a</sup>	Total count A		Yeasts B	
	R <sup>2</sup>	Log red. (CFU/ml) <sup>b</sup>	R <sup>2</sup>	Log red. (CFU/ml) <sup>b</sup>
0.073 A	0.950	1.18	0.975	1.20
0.165 B	0.980	1.98	0.962	1.88
0.255 C	0.991	2.39	0.991	2.47
0.349 C	0.987	2.68	0.986	2.70
0.451 C	0.979	2.94	0.965	2.71

<sup>a</sup> Comparing total count and yeasts for flow rates and times. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Log reduction after 30 min of UV treatment.

**Microbial reduction.** The initial microbial load for total microbial count and yeast count was in the range 5.49 to 5.62 and 5.46 to 5.56 log (CFU/ml) cycles, respectively. According to these data, the main microbial load corresponded to the yeasts. Also, no mold growth was observed during the entire study. Therefore, yeasts and molds are not mentioned as a group of microorganisms in this paper, just yeasts.

The first-order kinetics model was adequate for modeling the UV light microbial reduction at the selected flow rates (Figs. 3 and 4) in the range 75 to 450 kJ/m<sup>2</sup> (5 to 30 min). The increase of processing time (or dose or fluence) and flow rate increased the microbial reduction of total count (Fig. 3) and yeasts (Fig. 4). High correlation coefficients support the first-order modeling for both types of microbial loads (Table 2).

The  $D$ -value, commonly used to describe the decimal reduction effect in heat-treated food products (7), can also be used as  $D_{UV}$ -values to describe the effect of UV as a nonthermal treatment in microbial inactivation. The decimal reduction dose  $D_{UV}$  (kJ/m<sup>2</sup>) ( $D_{UV} = -2.303/k$ ) for both total microbial count and yeast count decreased as flow rate increased (Fig. 5). However, there is a limit to this microbial reduction since no difference ( $P \geq 0.05$ ) was observed for  $D_{UV}$ -values between flow rates 0.255, 0.349, and 0.451 liter/min (Table 2). On the other hand, the microbial reduction in total count was different from the yeast reduction ( $P \leq 0.05$ ). Even though the slopes showing decimal reduction of both total microbial count and yeast count look different in Figures 3 and 4, respectively, the increased flow rate did not decrease the  $D_{UV}$ -values (kJ/m<sup>2</sup>) appreciably. Therefore, no  $Z_{UV}$ -values were obtained since the log  $D_{UV}$ -values versus  $q$  representation did not follow a linear model. However, second-order polynomials (Fig. 5) for total microbial count (Equation 5) and yeast count (Equation 6) were obtained to describe the UV light processing of mango nectar:

$$D_{UV} = 3.60 \times 10^{-8}(q)^2 - 2.89 \times 10^{-5}(q) - 2.17 \times 10^{-4} \quad R^2 = 0.991, \quad (5)$$

$$D_{UV} = 4.09 \times 10^{-8}(q)^2 - 3.03 \times 10^{-5}(q) - 1.06 \times 10^{-4} \quad R^2 = 0.992. \quad (6)$$

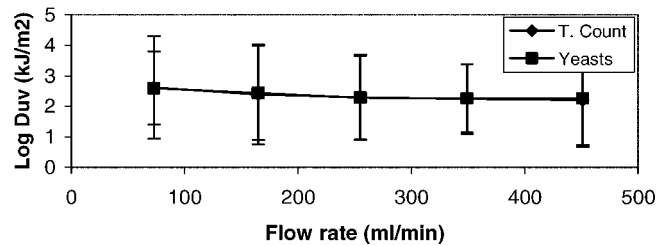


FIGURE 5. Yeasts and total count decimal reduction values for UV light-processed mango nectar.

The  $D_{UV}$ -values can also be represented as time taken to reduce 90% of the microbial load at constant flow rate. The  $D_{UV}$ -values in time can be converted to  $D_{UV}$ -values in kilojoules per square meter after multiplying by 15, or vice versa after dividing by 15.  $D_{UV}$ -values of 40.4 to 25.3, 16.6 to 9.2, and 16.8 to 6.3 min have been obtained for *S. cerevisiae*, *Escherichia coli*, and *Listeria innocua* in UV light-treated apple juice at flow rates ranging 0.073 to 0.451 liter/min (13). Lower  $D_{UV}$ -values for yeasts in mango nectar (yellow color) were observed than for yeasts in apple juice (brown color) at the same flow rates. Even though mango nectar is a turbid liquid, the UV light treatment improved the inactivation of microorganisms better than in apple juice, which is transparent but brown in color.

Fresh mango nectar was inoculated with *S. cerevisiae* to increase the initial total microbial count to 5.56 log (CFU/ml) cycles; as a result, more than 5 log were obtained initially in mango nectar to ensure the maximum log reduction. The maximum log reduction (CFU per milliliter) achieved was 2.94 and 2.71 for total count and yeasts, respectively, at the highest fluence (450 kJ/m<sup>2</sup> = 30 min) of UV light treatment at 0.451 liter/min (Table 2). This indicates that the required 5-log reduction of microorganisms by the U.S. Food and Drug Administration was not met to ensure a microbiologically safe product (32). However, with the good manufacturing practices recommended in the hazard analysis critical control point system (5), the process of obtaining mango nectar may render a fresh product with low microbial load, which can be further reduced with UV treatment. On the other hand, permitted antimicrobials, such as benzoic or sorbic acid, can be added to fresh mango nectar before UV treatment to delay spoilage during storage. Benzoic acid (up to 0.1%) is effective on some bacteria, yeasts, and molds at pH levels ranging from 2.5 to 4.5. Sorbic acid is used in the range 0.025 to 0.075% in fruit drinks to delay the growth of yeasts and bacteria (9). Hence, the addition of these antimicrobials to mango nectar before UV treatment could improve the shelf stability for longer periods at low temperature.

**PPO.** Figure 6 presents the remaining PPO activity in mango nectar after UV light treatment. The initial PPO activity was  $76.8 \pm 37.8$  EAU. The highest PPO reduction after UV light application was observed after the lowest treatment time (5 min). Since the maximum average temperature was  $29.9 \pm 1.6^\circ\text{C}$  after 30 min of treatment at all flow rates, it is possible UV light was absorbed into the organic molecules (20). This absorbed energy can lead to

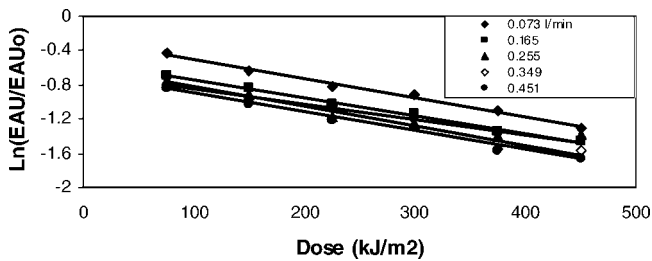


FIGURE 6. First-order kinetics modeling of polyphenoloxidase activity in UV light-treated mango nectar at different flow rates.

photoinactivation of proteins such as PPO. UV energy is absorbed by conjugated double bonds (excited) and then reacts with  $O_2$  to produce single bonds (22). Therefore, UV light treatment can change molecules with conjugated double bonds and cause chemical changes in their properties.

A significant difference ( $P < 0.05$ ) was observed in the remaining PPO activity at different flow rates (Table 3). The higher the flow rate, the higher the PPO reduction after 30 min of UV light treatment; however, no significant difference ( $P > 0.05$ ) was observed in the remaining PPO activity after treatment between 25 and 30 min.

$D_{UV}$ -values were computed from the first-order kinetics modeling and ranged from 152 to 199 min (2,280 to 2,991  $kJ/m^2$ ), but no significant differences were observed ( $P \geq 0.05$ ) among values (Table 3). A slope and intercept of  $-3.22 \times 10^{-4}$  and 2.32, respectively, were computed from the log  $D_{UV}$  versus flow rate representation ( $R^2 = 0.847$ ), but high variability in  $D_{UV}$ -values was observed (Fig. 7). A  $Z_{UV}$ -value of 3.105 liters/min was computed from the slope. In thermal processing, the decimal reduction time ( $D$ ) and  $Z$ -values found on the thermal death time curve are commonly higher for enzyme inactivation than for microbial inactivation (7). The same behavior holds for  $D_{UV}$ -values for microorganisms (Fig. 5) and enzymes (Fig. 7) in UV light-processed mango nectar.

**Color.** Mango nectar studied was a bright yellow, turbid liquid. The initial  $L^*$ ,  $a^*$ , and  $b^*$  color parameters were  $44.7 \pm 0.6$  (light),  $3.0 \pm 0.3$  (red), and  $34.7 \pm 1.1$  (yellow), respectively. Therefore, the yellow color prevailed with a tint of red. Figure 8 illustrates the total change in color of UV light-treated mango nectar. According to instrument measurements, the main change in color was observed dur-

TABLE 3. First-order kinetics modeling parameters for PPO activity in UV light-processed mango nectar at different flow rates

Flow (liter/min) <sup>a</sup>	$D_{UV}$ ( $kJ/m^2$ )	EAU (%) <sup>b</sup>	$R^2$
0.073 A	$2,991 \pm 120$	$27 \pm 3$	0.939
0.165 B	$2,709 \pm 450$	$23 \pm 8$	0.869
0.255 BC	$2,709 \pm 75$	$25 \pm 6$	0.792
0.349 C	$2,280 \pm 180$	$21 \pm 5$	0.919
0.451 C	$2,350 \pm 165$	$19 \pm 4$	0.839

<sup>a</sup> Comparing remaining PPO for flow rates and times. Means with the same letter in the same column are not significantly different ( $P > 0.05$ ).

<sup>b</sup> Remaining PPO after 30 min (450  $kJ/m^2$ ) of UV light treatment.

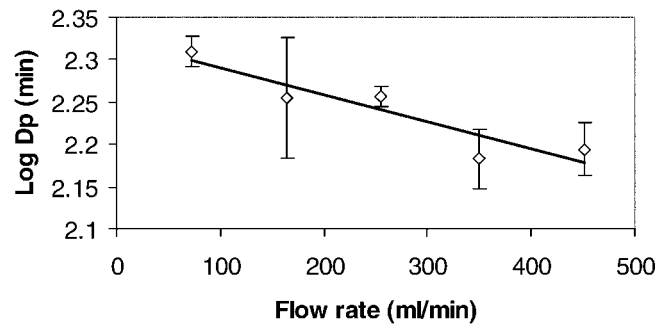


FIGURE 7. Decimal reduction values ( $D_p$ ) for PPO activity in UV light-processed mango nectar.

ing the first 5 min of treatment at all flow rates. However, no change in yellow was noticed in the final product. No significant differences ( $P > 0.05$ ) were observed for  $\Delta E^*$  values between flow rates and only the 0- and 30-min treatments were different from other treatment times for both  $a^*$  and  $b^*$  color parameters ( $P < 0.05$ ). Averages of  $3.9 \pm 1.8$ ,  $46.4 \pm 1.9$ ,  $2.5 \pm 0.3$ , and  $32.6 \pm 3.1$ , for all flows and process times, were observed for  $\Delta E^*$ ,  $L^*$ ,  $a^*$ , and  $b^*$ , respectively. Carotenenes (ranging in color from yellow to red for fruits) become unstable when exposed to light, heat, acid medium, and air or oxygen (11). Of these, UV light is the most harmful factor in color deterioration. Organic colorants may undergo photodegradation due to the absorption of energy by double bonds; the excited double bond reacts with oxygen, which leads to a single bond. The addition of OH to each end of the double bond may lead to the loss of color (22). However, the yellow color of the UV-treated mango nectar remained the same after treatment even under the highest processing conditions. Hence, UV light barely affected the color of mango nectar, since initial color parameters  $L^*$  (44.7),  $a^*$  (3.0), and  $b^*$  (34.7) changed to 46.4 (lighter), 2.5 (less red), and 32.6 (less yellow), respectively.

**Shelf life of mango nectar: microbial reduction.** Figures 9 and 10 illustrate the total count and yeasts in stored UV light-treated ( $3^\circ C$ ) mango nectar. The initial total count and yeasts were in the range 4.54 to 4.61 and 3.0 to 3.28 log (CFU/ml) cycles, respectively. The total microbial count was reduced in the range 3.82 to 4.13 and 2.86 to 3.41 log (CFU/ml) cycles after 15 min of UV treatment at 0.073 and 0.451 liter/min, respectively. On the other hand, yeasts were reduced in the range 2.46 to 2.62 and 1.82 to

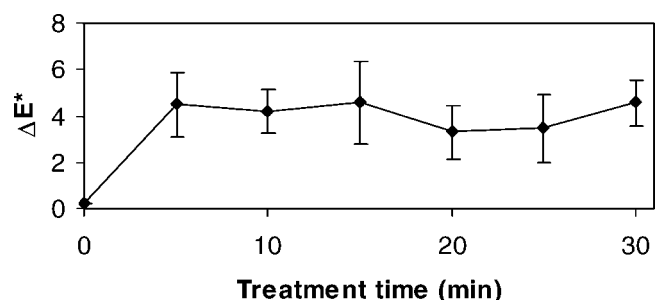


FIGURE 8. Total color difference of UV light-treated mango nectar at different flows and times.

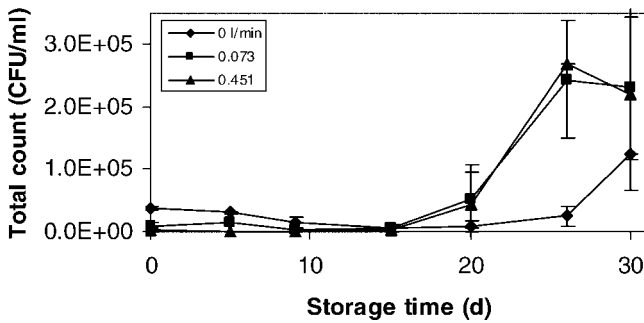


FIGURE 9. Total microbial count growth of UV light-processed mango nectar stored at 3°C.

1.97 log (CFU/ml) cycles under the same time and flow rate conditions. Evaluation of these data indicate that only 1.58- and 1.38-log reductions were observed for total count after treatment at 0.073 and 0.451 liter/min, respectively, and only 1.62- and 1.26-log reductions were observed for yeasts at the same flow rates, respectively. Therefore, the 5-log reduction recommended by the U.S. Food and Drug Administration for juices (32) cannot be accomplished at 0.073 or 0.451 liter/min with 15 min of UV treatment. The total microbial count (Fig. 9) and yeasts (Fig. 10) were barely maintained constant for 20 days of storage. However, a rapid microbial growth was observed for both types of microbial counts from this time in UV light-treated mango nectar. A higher microbial growth was observed in UV light-processed mango nectar than in nonprocessed mango nectar from 20 days of storage. Stevens et al. (31) pointed out that cells may be photoreactivated when they are exposed to wavelengths higher than 330 nm. However, UV light-treated mango nectar was stored in the darkness. Microbiologically safe mango nectar can be obtained with (i) the addition of some antimicrobial agents, (ii) increase of treatment time, or (iii) increase of UV unit's natural temperature as an additional hurdle, but no higher than pasteurization temperatures since the process is supposedly nonthermal.

**PPO and color.** Figure 11 presents the remaining PPO activity of mango puree during storage. The initial PPO activity of  $1,064 \pm 90$  EAU in mango nectar was reduced to  $74 \pm 1\%$  and  $60 \pm 3\%$  after 15 min of UV treatment at 0.073 and 0.451 liter/min, respectively. The remaining enzyme activity in mango nectar at the studied flow rates

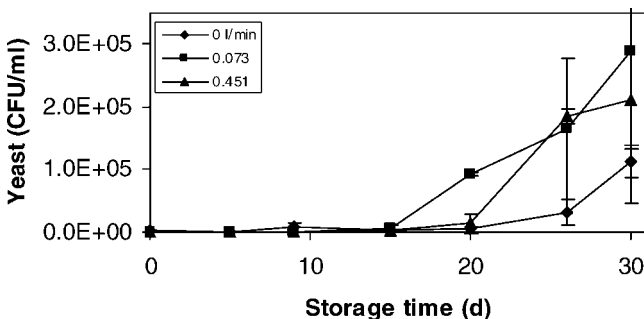


FIGURE 10. Yeast growth of UV light-processed mango nectar stored at 3°C.

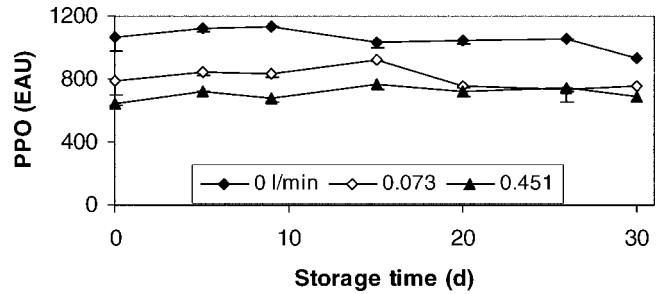


FIGURE 11. Polyphenoloxidase activity in UV light-treated mango nectar stored (3°C) in glass tubes.

was significantly different ( $P < 0.05$ ), and the remaining enzyme activity in mango nectar stored 30 days was only different from enzyme activity at other storage times. Averages of the remaining enzyme activity in mango nectar over the entire storage period were  $1,055 \pm 32$ ,  $803 \pm 32$ , and  $710 \pm 37$  EAU for 0, 0.073, and 0.451 liter/min, respectively (Fig. 11). Therefore, no substantial PPO activity changes in mango nectar were observed during the entire storage. However, to delay the reaction of the remaining enzyme activity, the addition of antibrowning agents and lowering of pH is recommended to help increase the storage stability of UV light-treated mango nectar.

Figure 12 illustrates the total change in color of mango nectar stored at 3°C. The initial  $L^*$ ,  $a^*$ , and  $b^*$  color parameters observed before UV treatment were  $41.0 \pm 0.3$ ,  $5.1 \pm 0.1$ , and  $34.9 \pm 0.4$ , respectively. Values of  $44.1 \pm 2.5$ ,  $4.4 \pm 0.3$ , and  $29.2 \pm 3.7$  were observed after UV light treatment for  $L^*$ ,  $a^*$ , and  $b^*$ , respectively. However, no change in orange-yellow color was noticed in UV light-treated mango nectar when compared with non-UV light-treated mango nectar. Untreated mango nectar started to darken after 15 days of storage. The  $\Delta E^*$  total change in color for untreated mango nectar was different from the  $\Delta E^*$  values of UV light-treated mango nectar ( $P < 0.05$ ). UV light-treated mango nectar at 0.073 and 0.451 liter/min maintained yellow and orange-yellow colors, respectively, for 26 days of storage at 3°C. The average  $L^*$  parameters observed in mango nectar for the entire storage period were 37.0, 38.5, and 41.2 for 0, 0.073, and 0.451 liter/min of UV light treatment, respectively. Therefore, browning in UV light-treated mango nectar increased as flow rate decreased. On the other hand, averages of  $a^*$  and  $b^*$  color parameters for mango nectar, independently analyzed, did

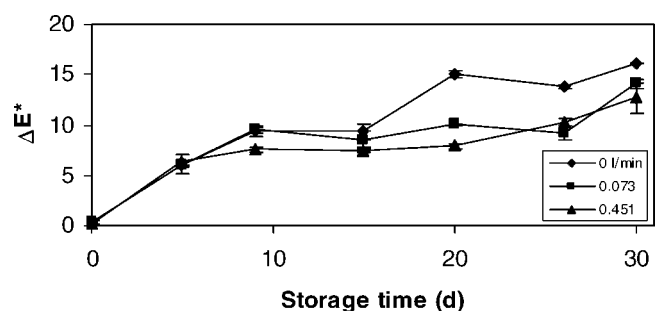


FIGURE 12.  $\Delta E^*$  color changes of UV light-treated mango nectar stored (3°C) in glass tubes.

not show significant differences ( $P > 0.05$ ) between flow rates. Therefore, the main change in color parameters observed was lightness.

Reduction of *S. cerevisiae* in UV light-treated mango nectar followed a first-order kinetics.  $D_{UV}$ -values computed from the constant rate ( $k$ ) were obtained for both total microbial count and yeast count. These  $D_{UV}$ -values can be used to predict the log reduction at each flow rate. However,  $Z_{UV}$ -values were not obtained since the log  $D_{UV}$  versus flow rate model did not follow a straight line.  $D_{UV}$ -values for PPO activity were also obtained along with a  $Z_{UV}$  of 3.1 liter/min.  $D_{UV}$ -values for the remaining enzyme activity were higher than for the microorganisms. No more than 2.94- and 2.71-log reductions (CFU per milliliter) were obtained for total microbial count and yeast count, respectively, at the flow rates and times used in this research. No substantial change in color was observed after UV light treatment of mango nectar.

The initial microbial load of the stored mango nectar was reduced in the range 1.38 to 1.58 and 1.25 to 1.62 log (CFU/ml) cycles for total count and yeast count, respectively, after UV light treatment. Shelf life of the mango nectar lasted for 20 days with almost no microbial growth. UV-treated mango nectar at 0.073 and 0.451 liter/min maintained yellow and orange-yellow colors, respectively, after 26 days of storage. PPO activity remained barely constant after 30 days of storage. However, the lower the flow rate during UV light treatment, the higher the browning of mango nectar during storage. Increased treatment time is advisable to further reduce the initial microbial load. Also, the addition of antimicrobial and antibrowning agents is advisable before UV treatment to increase shelf life of UV light-treated mango nectar stored at refrigeration temperatures.

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