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Removing patulin from apple washing water by UV radiation

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ABSTRACT

Based on their importance in UV radiation reactions, six main factors (UV wavelength, radiation time, radiation intensity, initial patulin concentration, pH, and depth of solution) were chosen to investigate their effects on the photodegradation of patulin in apple washing water (AWW). UV radiation with 254 nm lamp could significantly degrade patulin in AWW, while no role on patulin with 365 nm lamp. Increasing the radiation time (0–40 min) could significantly reduce patulin in AWW, which followed a pseudo-first-order kinetic model. Increasing radiation intensity could also significantly decrease patulin in AWW by obeying a zero-order kinetic model. No visible effect of initial patulin concentration (25–500 μg/L) on its photodegradation was observed. Patulin was relatively instable in acidic or alkalic condition, which was beneficial to increase its photodegradation. Under the same radiation conditions, increasing the depth of solution obviously decreased the photodegradation efficiency of patulin. These results will be helpful in developing UV photodegradation equipment of patulin and treating AWW.

Keywords: Patulin; UV photodegradation; Apple washing water (AWW); Influencing factors; Kinetic model

1. Introduction

Patulin is a secondary metabolite mainly produced by *Penicillium expansum*, which is frequently found as a post-harvest contaminant in many fruits and their products [1,2]. It is a natural human toxin and therefore has genotoxicity, teratogenicity, mutagenicity, embryotoxicity, and carcinogenicity [3–5]. The International Agency for Research on Cancer (IARC) has classified patulin as Group 3 or as "not carcinogenic to humans." Many countries, including EU and USA, have recommended the maximum level of patulin permitted in fruit juice is 50 μg/kg [6,7].

CAC and FAO have recommended that juice companies should adopt good agricultural practices, good manufacturing practices, and hazard analysis and critical control points to reduce the contamination of patulin throughout the productive chain of fruit juices [8,9]. And physical (filtering, adsorption, and UV radiation), chemical (ozone, ammonia, and sulfur dioxide), and biological (yeast fermentation) methods have been studied to reduce the level of patulin during fruit juice processing [5]. Among these detoxification methods, UV radiation has been widely studied to degrade patulin in liquid foods or mode systems in recent years [10–14]. UV radiation can effectively degrade patulin in apple-based foods and its degradation follows the first-order reaction model [10,11,14]. Compared with other methods, UV radiation has many advantages, such as high detoxifying efficiency, low cost, easy operation, disinfecting role, and without residual of hazardous substances [10,15,16]. While the detoxifying efficiency of patulin by UV radiation can be affected by many factors, such as UV intensity, exposure time, UV wavelength, juice matrix (pH, Brix, glucose, sucrose, ascorbic acid, tannic acid, and color), temperature, and initial patulin concentration in juices [13,17].

Patulin is easily soluble in water, so washing (especially pressure spraying) has been shown to be effective in removing

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patulin from fruits [18–20]. During the washing process, patulin in apples comes into water, which cannot be reused or discharged into environment directly before detoxifying it. So the objective of this study is to reduce the patulin in apple washing water (AWW) by UV radiation and to explore the factors influencing photodegradation efficacy of patulin in AWW.

2. Materials and methods

2.1. Chemicals and materials

Patulin standard (>98%) was purchased from Sangon Biotech (Shanghai, China). HPLC-grade acetonitrile and formic acid were purchased from Yuwang company (Dezhou, China) and Kemiou chemical reagent company (Tianjin, China), respectively.

2.2. UV radiation treatment

The UV radiation unit self-made includes UV lamps with different wavelength (Cnlight lamp, 254 nm, 36 W, pipe diameter 17 mm, and length 411 mm; Philips lamp, 365 nm, 36 W, pipe diameter 17 mm, and length 410 mm), glass plate with diameter of 11.5 cm and height of 2.5 cm, and an adjustable speed magnetic stirrer (Fig. 1).

Stock solution of patulin (5 μg/mL) was prepared with purified water and stored at 10°C in refrigerator for further use. Patulin solutions with different concentrations obtained by diluting the stock solution with AWW were used in the UV photodegradation experiments.

Effects of UV wavelength on photodegradation of patulin were evaluated using the two types of UV lamp earlier mentioned, while the UV lamp with 254 nm was used in all other remaining tests. Effects of UV radiation intensity on photodegradation of patulin were carried out at 1.37, 1.90, 2.83, 4.31, 5.80, and 6.20 mW/cm^2, respectively, which were measured by placing the probe of the UV light meter (Model UV-254A) on the horizontal position with the upper surface of treated water, and each UV intensity on the surface of treated water was the average value of three repetitions. The different UV radiation intensities were obtained by adjusting the distances between UV lamp and upper surface of treated water, which corresponded to the distances of 18.0, 12.5, 8.0, 5.0, 2.0, and 0.5 cm, respectively. Effects of UV radiation time on photodegradation of patulin were measured at 10–40 min with an interval of 10 min and the radiation time of 10 min was used in all other tests. Effects of initial patulin concentration in AWW were evaluated at 25, 50, 100, 150, 200, 250, 300, 400, and 500 μg/L, respectively. Impacts of solution pH on photodegradation of patulin were assessed at 3.08, 3.50, 4.01, 4.56, 5.02, 6.28, 7.86, and 10.10, respectively. The AWW itself exhibited a pH value of 6.28, which was treated as one of the eight cases. The other seven initial pH values were obtained by adjusting original pH (6.28) of AWW with 0.1 mol/L of HCl or NaOH solution. Impacts of water depth on photodegradation of patulin with and without stirring at 200 rpm were assessed at seven different depths (2.4, 4.8, 7.2, 9.6, 12.0, 14.4, and 19.2 mm), which were obtained by changing the volume of water in the same glass plate. All experiments were carried out at room temperature with continuous stirring at 200 rpm.

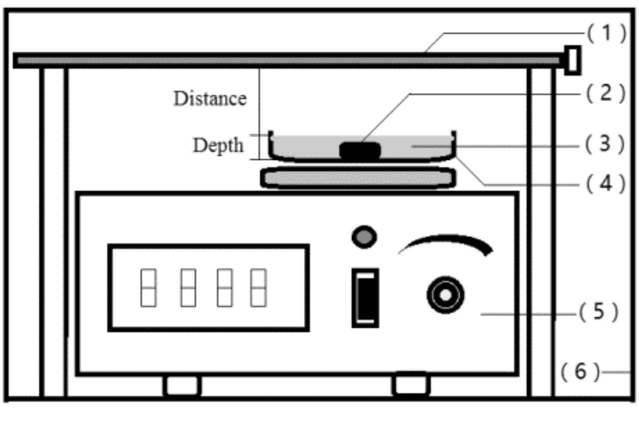

Fig. 1. UV radiation unit (1) UV lamp, (2) stirring rod, (3) AWW containing patulin, (4) glass plate, (5) magnetic stirrer, and (6) shield.

2.3. Determination of patulin concentration by HPLC

Patulin in purified water was determined using Shimadzu LC-20A HPLC system and Waters XBridge C18 column (100 × 4.6 mm, i.d. 3.5 μm). 20 μL sample was injected and 0.1% of formic acid solution/acetonitrile (95:5, v/v) was used as isocratic mobile phase with a flow rate of 0.75 mL/min at 30°C. The detector wavelength was set at 276 nm. For this method, the limit of detection was 2.4 μg/L and the limit of quantification was 8.5 μg/L. Its average recovery was 97.8% and the relative standard deviation (SD) was 1.3%.

2.4. Statistical analysis

Each analysis was carried out in triplicate for each sample and all values were expressed as means ± SD. The difference between control and UV-treated groups was compared by the Student's *t*-test using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The results were considered significant if the *P* values were less than 0.05.

3. Results and discussion

3.1. Effects of UV wavelength and radiation time

UV radiation is classified into three types according to its wavelength, that is, UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). Their differences are in their biological activity and the extent to which they can penetrate substance. In the reported literatures, most studies in UV radiation detoxification were carried out at 254 nm, which was the germicidal wavelength [10–13]. While a multiwave emitting lamp was used to degrade patulin at all the wavelengths from 255 to 355 nm and reduce the microbial burden at 255 nm [12]. In addition, the 222 nm wavelength possessed the highest efficiency for patulin reduction in apple juice when compared with the reductions at 254 and 282 nm, that is, 222 > 282 > 254 nm [21]. In this study, UVA (365 nm) and UVC (254 nm) were chosen to degrade patulin in AWW, which were also usually used in the food industry to detoxify mycotoxins and other hazardous substances in foods and water [22–24]. Effects of UV wavelength on patulin degradation are illustrated in Fig. 2. UVC (254 nm) radiation significantly reduced the patulin content in AWW ($P < 0.01$), while UVA (365 nm) had not any role on it ($P > 0.05$) in the time range from 10 to 40 min. Patulin has several absorption bands ranged from 255 to 350 nm with different molar extinction coefficients (ε_λ), which may cause the photochemical degradation of patulin. Patulin has higher ε_λ at UVC than UVA, which is the reason why UVC has a higher detoxifying efficiency than that of UVA [12]. Therefore, the results in this study further verified that UVC can significantly degrade patulin in AWW compared with UVA ($P < 0.01$), which was used to study the degradation of patulin in AWW in the next work.

For the UV photodegradation of patulin at 254 nm, UV radiation time is a very important factor influencing the degradation of patulin in AWW. Patulin in AWW was degraded from initial concentration of 200.52 ± 2.21 μg/L to 44.14 ± 0.40 μg/L ($P < 0.01$) within 20 min at UV intensity of 4.31 mW/cm^2 and reduced by 77.99%. After being radiated for 40 min, patulin in AWW was decreased to 12.55 ± 0.44 μg/L with a degradation efficiency of 93.74%. The decomposition of patulin in AWW at different UV radiation times obeyed the pseudo-first-order kinetic model, that is, $[C]_t = [C]_0 \exp(-kt)$ or $\ln([C]_t/[C]_0) = -kt$, where $[C]_t$ is the concentration of patulin at UV radiation time of t and $[C]_0$ is the concentration at time of 0, and k is the first-order rate constant. According to the kinetic equation above-mentioned, the rate constant k is 0.067 min^{-1} and the corresponding correlation coefficient (R^2) is 0.9980. The similar results had been obtained using 253.7 nm UV lamp to degrade patulin in model solution, apple cider and apple juice [11]. They achieved 56.5%, 87.5%, and 94.8% reduction of patulin, respectively, in the media mentioned above initially spiked by 1.0 mg/L of patulin with UV exposure for 40 min at UV intensity of 3.00 mW/cm^2.

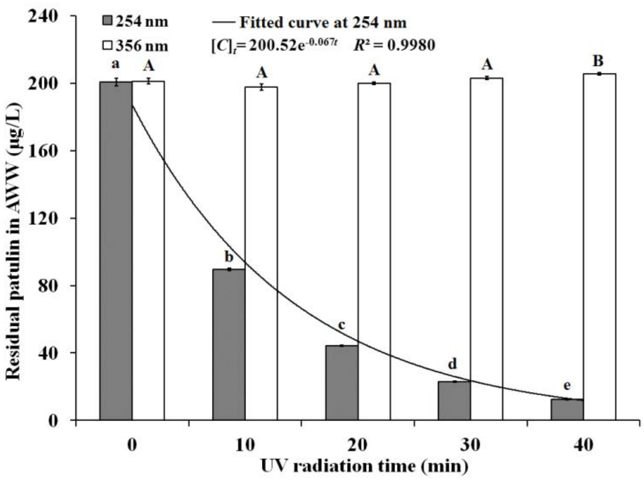

Fig. 2. Effects of UV wavelength and radiation time on photodegradation of patulin in AWW. UV radiation conditions: radiation intensity was 4.31 mW/cm^2, pH of AWW was 6.28, solution depth was 2.4 mm, and stirring at 200 rpm. (Different letters indicate the significant difference at $P < 0.01$ level between UV radiation times and the fitted curve equation is $[C]_t = 200.52 \exp(-0.067t)$, $R^2 = 0.9980$).

3.2. Effect of UV radiation intensity

UV radiation intensity is a very important factor influencing patulin degradation. It was reported that UV exposure of 14.2–99.4 mJ/cm^2 resulted in a significant and nearly linear decrease in patulin contents, and patulin levels decreased by 9.4%–43.4% in the range of UV doses tested [10]. The similar results were obtained using the UV doses ranged from 0 to 8 J/cm^2 to degrade patulin in a model apple juice system and in apple juice [13]. In this study, the effects of UV radiation intensity on patulin decomposition were conducted under six levels by adjusting the distances between UV lamp and upper surface of treated AWW. The results are shown in Fig. 3. Increasing UV intensity significantly improved the decomposition efficiency of patulin in AWW ($P < 0.01$). At the UV intensity of 4.31 mW/cm^2 for 10 min of exposure, the patulin was reduced from 199.38 ± 4.10 μg/L to 91.29 ± 2.64 μg/L, decreased by 54.22%, and to 38.35 ± 1.21 μg/L at 6.20 mW/cm^2 with a degradation rate of 80.77%. The UV radiation intensity was directly proportional to the decomposition of patulin in AWW ($R^2 = 0.9950$) and it followed a zero-order kinetic model (Fig. 3). Therefore, reducing the distance between UV lamp and upper surface of treated samples contributes to improve the photodegradation efficiency of patulin with the same UV lamp.

3.3. Effects of initial patulin concentration

Based on the high contamination rates and different levels of patulin in AWW, the initial patulin concentration in AWW was set in the range of 25–500 μg/L. The effects of initial patulin concentration on its decomposition are presented in Fig. 4. The degradation efficiencies of patulin in AWW with various concentrations ranged from 25 to 500 μg/L were almost similar ($P > 0.05$), which were in the range from 55.17% to 61.05% within 10 min of UV radiation. Therefore, the initial patulin concentration does not have a

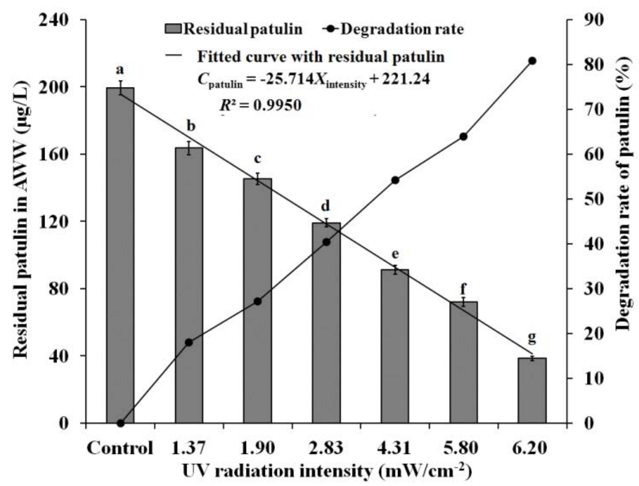

Fig. 3. Effects of UV radiation intensity on photodegradation of patulin in AWW. UV radiation conditions: radiation time was 10 min, pH of AWW was 6.28, solution depth was 2.4 mm, and stirring at 200 rpm. (Different letters indicate the significant difference at $P < 0.01$ level between UV radiation intensities).

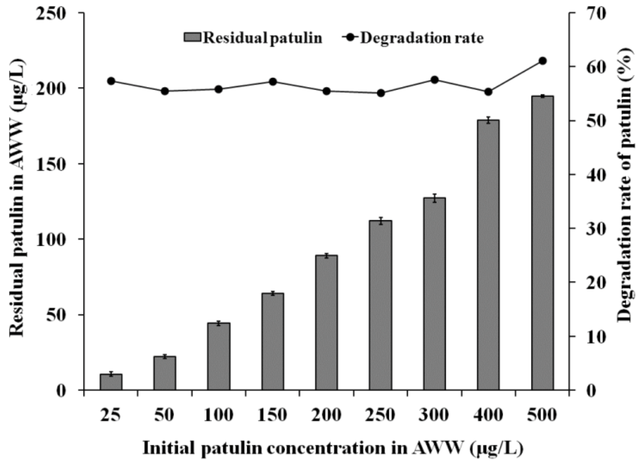

Fig. 4. Effects of initial patulin concentration on UV photodegradation efficiency of patulin in AWW. UV radiation conditions: radiation intensity was 4.31 mW/cm², radiation time was 10 min, pH of AWW was 6.28, solution depth was 2.4 mm, and stirring at 200 rpm.

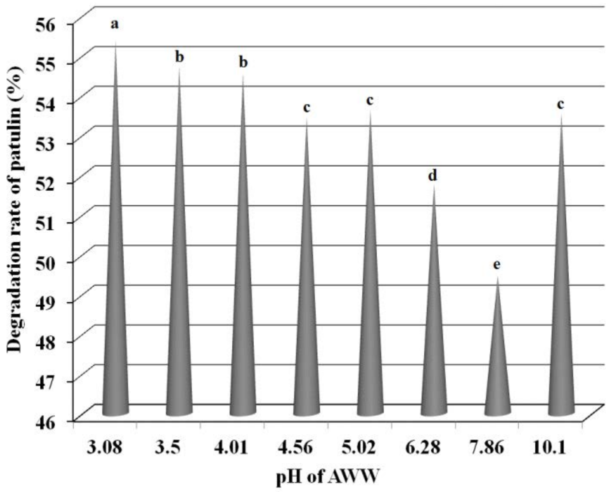

Fig. 5. Effects of pH on UV photodegradation of patulin in AWW. UV radiation conditions: radiation intensity was 4.31 mW/cm², radiation time was 10 min, solution depth was 2.4 mm, and stirring at 200 rpm. (Different letters indicate the significant difference at $P < 0.05$ level between different pHs of AWW).

significant effect on its degradation, which is consistent with the reported results [13].

3.4. Effects of solution pH

Results from many reported literatures showed that patulin was stable in an acidic environment and was not destroyed during thermal processing [25,26], while it was instable at high pH [27]. The half-life of patulin held at 25°C with pH 6.0 and 8.0 were 55 and 2.6 d, respectively [28]. However, its stability was reduced at acidic pH, especially the presence of ascorbic acid [29]. Tikekar et al. explored the effect of pH (3.0–3.6) on UV induced degradation of patulin (1,000 μg/L) and found that no significant differences in degradation efficiency of patulin in apple juice and model system due to the slight difference in pH [13]. While some researchers reported the photodegradation of patulin was higher at low pH (4.0 and 3.0) than at high pH (7.0 and 6.0) [12,14]. In this study, photodegradation efficiencies of patulin in acidic and alkalic conditions were all significantly increased ($P < 0.05$) and it was relatively stable to UV radiation at nearly neutral pH (Fig. 5). The reason is that UVC radiation may obviously increase the content of OH free radical whether in acidic or alkalic conditions, which is a strong oxidant and accelerates the degradation of patulin in AWW [30,31]. The reasons will need to be further explored in the future work.

3.5. Effects of solution depth

It is well-known that UV light has a low power of penetration, especially in the ranges of UVC. So the depth or thickness of radiated sample is also an important factor influencing the photodegradation of patulin. It was reported that the UV radiation (255–355 nm) entering the patulin solution was absorbed for depths of much less than 1 mm [17]. Ibarz et al. modeled the photodegradation of patulin with a solution height of 2.4 cm and found that less energy reached the bottom than the surface [12]. Fig. 6 depicts the effects of AWW depth on the photodegradation of patulin. The results verified the UV radiation has a low power of penetration and the degradation efficiency of patulin was significantly decreased with the increase of solution depth ($P < 0.01$). For the solution depth of 2.4 mm, the patulin was decreased from 212.50 ± 1.44 μg/L for the control to 93.80 ± 1.78 μg/L and reduced by 55.86% ($P < 0.01$) at the radiation intensity of 4.36 mW/cm² for 10 min. While for the depth of 14.4 mm, it was decreased to 106.54 ± 1.03 μg/L with a reduction of 49.86% at the same conditions. Based on the low penetration power of UVC, it is necessary to reduce the depth of radiated solution to the reasonable level for increasing the degradation efficiency of patulin.

Based on the low power of penetration of UVC radiation, we considered that it was necessary to stir the AWW using a stirrer for improving the photodegradation of patulin. While the results were not same as what we thought (Fig. 7). Generally, the photodegradation efficiencies of patulin were reduced with the increase of AWW depth with or without stirring. When the depths of AWW were less than 9.6 mm, the degradation efficiencies of patulin without stirring were greater than those of patulin with stirring at 200 rpm under the other same UV radiation conditions. While the depths of AWW were greater than 14.4 mm, the opposite results were obtained. The experiment process was investigated carefully and found that the stirring rod used to stir AWW has a diameter of 7.0 mm. When the depths of AWW were less or slightly greater than the diameter of the rod (such as the depth of 9.6 mm), the rod could not completely agitate the water, and the presence of rod restricted the UV photodegradation of patulin. As the depths of AWW were far greater than the diameter of the rod, the role of rod in improving the photodegradation efficiencies of patulin could be obtained.

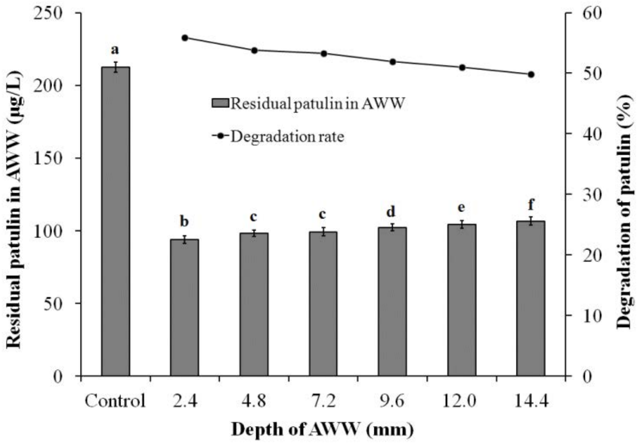

Fig. 6. Effects of AWW depth on UV photodegradation of patulin in AWW. UV radiation conditions: radiation intensity was 4.36 mW/cm, radiation time was 10 min, pH of AWW was 6.28, and without stirring at 200 rpm. (Different letters indicate the significant difference at $P < 0.01$ level between different depths of AWW).

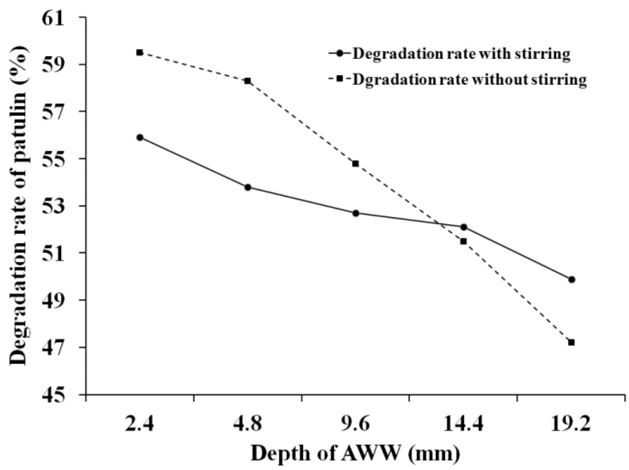

Fig. 7. Effects of stirring on UV photodegradation of patulin in AWW. UV radiation conditions: radiation intensity was 4.36 mW/cm^2, radiation time was 10 min, pH of AWW was 6.28, and with or without stirring at 200 rpm.

4. Conclusions

UV radiation could effectively degrade patulin in AWW and many factors impacted the UV photodegradation of patulin. UVC could quickly degrade patulin while UVA had no any role on it. The photodegradation of patulin in AWW was time-dependent and followed the pseudo-first-order kinetic model. Increasing the UV radiation intensity obviously improved the decomposition of patulin, which obeyed a zero-order kinetic model. The initial patulin concentration (25–500 μg/L) did not have a visible influence on its photodegradation. Solution pH showed profound impacts on photodegradation of patulin. Both acidic and alkalic environments could all reduce the stability of patulin in AWW, which contributes to increasing the photodegradation efficiencies of patulin. Based on the low penetration of UVC, the depth of AWW should be decreased to a reasonable level for effective photodegradation of patulin. Stirring might greatly improve the UV photodegradation of patulin when the depth of water was far greater than the diameter of the stirring rod, which could completely agitate the solution. The pH of AWW was indirectly responsible for the reduction in photodegradation of patulin in it.

Acknowledgments

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