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Glow Discharge Plasma Efficiently Degrades T-2 Toxin in Aqueous Solution and Patulin in Apple Juice

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Abstract

The degradation of T-2 toxin in aqueous solution and patulin in apple juice at different conditions by glow discharge plasma (GDP) was investigated. High performance liquid chromatograph (HLPC) was used to analyze the toxins concentration which was changed by treatment time. The results showed that GDP treatment could rapidly and effectively degrade T-2 toxin in aqueous solution and patulin in apple juice. The higher the initial toxin concentrations, the higher treatment efficiency could be achieved within the same time. The degradation rates of T-2 toxin at any different initial concentration were all up to 30% after 8 min, whilst no T-2 toxin was detected after 40 min. The toxin removal rate was faster at a relatively higher acidity and alkalinity levels. The Fe^{2+} and H_2O_2 exhibited strong catalysis ability to the degradation reactions. The values of pH in the degraded solution were decreased rapidly due to the formation of carboxylic acids. Afterwards, the values were increased as carboxylic acids were decomposed into CO_2 and H_2O. The dynamics equation curve is most appropriate and confirms that the degradation reaction of T-2 toxin by GDP belonged to the first order kinetics reaction, which could be expressed as $\ln (C_0/C_t)=kt$. Quality evaluation of the apple juice indicated that GDP treatment within 10 min had little effects on quality of apple juice.

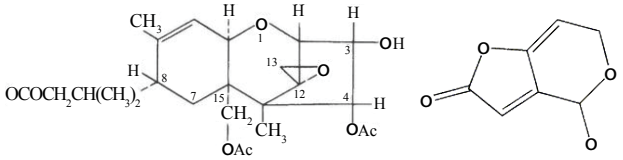
Keywords: T-2 Toxin; Patulin; Glow discharge plasma; Degradation

Introduction

T-2 toxin, produced by various *Fusarium* spp. (*F. sporotrichiodes*, *F. poae*, *F. equiseti* and *F. acumimatum*), is one of the most toxic mycotoxins belonging to the type Atrichothecenes. This metabolite possesses a characteristic 12, 13-epoxytrichothecene-9-ene ring structure, as shown in Figure 1, which is responsible for the toxicity [1]. Chronic and acute toxic effects of T-2 toxin in animals such as vomiting, weight loss, nausea, diarrhea, inflammation and infertility has been reported [2]. As early as in 1973, FAO designated it as the most dangerous natural food pollutant together with aflatoxin [3]. As it has stable chemical structure, T-2 toxin has been reported to be the most difficult toxin to be degraded among the trichothecene mycotoxins [4]. Although there are many reports about degradation of T-2 toxin, such as the method of chemistry [5,6], physical [7,8] and biology [9,10], they did not get satisfactory results. The toxicity of transformation products had been lowered after heat treatment, but there was no phenomenon about the degradation of T-2 toxin [8]. Similarly, the obvious reduction of T-2 toxin amount in grains did not appear after treatment of γ-ray and electron beam [11]. The toxicity of T-2 toxin could be lowered by treatment of NaClO solution in NaOH, but it could result in the Chloride residue [12,13]. *Baccharis* spp. can oxidize T-2 toxin into 3'-OH T-2 or 3'-OH HT-2 through the reaction of hydroxylation [14], but it was not certain if the products has the toxicity. Again, *Trichoderma harzianum* could reduce the acumination of T-2 toxin in crop by inhibiting the growth of pathogens, but it did not degrade T-2 toxin and patulin directly [15].

Patulin is a mycotoxin produced by certain species of Aspergillus and *Penicillium expansum*. It is mainly found in apples and products derived from apple, particularly in apple juice. Patulin contamination is a health hazard to humans and reduces commodity values. Exposure to this mycotoxin is associated with a broad range of adverse effects, including gastrointestinal diseases and potential for carcinogenicity and genotoxicity, immunotoxicity and neurotoxicity have been observed [16,17]. The Food and Agriculture Organization (FAO) of the United Nations has also recommended adoption of the Hazard Analysis and Critical Control Points system (HACCP) so as to guarantee the control of patulin in apple juice [18]. Joint FAO/WHO Expert Committee on Food Additives (JECFA) has lowered the provisional maximum tolerable daily intake (PMTDI) for patulin from 1 to 0.4 mg/kg body mass/day based on a no observed effect level (NOEL) of 43 mg/kg body mass/day and the use of a 100-fold safety factor [19]. Many Methods including physical methods of UV illumination, thermal and ultrasonic treatments, as well as chemical and biological methods had been used to treat the T-2 toxin and Patulin. But the effects were not obvious [20].

Therefore, effective, inexpensive and easily operated technologies are needed for treatment of T-2 toxin and patulin and also to eliminate



T-2 toxin Patulin

Figure 1: The chemical structure of T-2 toxin and patulin.

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the hazards of T-2 toxin and patulin through degradation, thereby enhancing food and feed safety.

Glow discharge plasma (GDP) is a novel kind of electrochemical process in which plasma is sustained by dc glow discharges between a pointed electrode and the surface of the liquid electrolyte [21]. The feature of GDP is that various active species such as hydrogen peroxide and hydroxyl radicals were formed when discharges take placed. These highly activated species can diffuse into the solution to oxidize the organic molecules in water. The mechanism in the production of plasma has been studied [22-25]. GDP could degrade most organic molecules to the production of CO_2 and H_2O. Recently, more studies have been conducted on the degradation of certain target organic compounds in water by using GDP [26-29]. The results reveal that GDP was promising in terms of the degradation of toxic substances. Although much attention has been focused on the degradation of T-2 toxin and patulin; yet the information of how GDP degrade them is unavailable. The purpose of this study is to investigate the effectiveness of GDP on the degradation of T-2 toxin in aqueous solution and patulin in apple juice at different conditions. The degradation kinetic curves and kinetic models were also evaluated. The T-2 toxin and patulin concentration and degradation products were studied using HPLC.

Experimental Methods

The main parts of experimental apparatus consisted of a high-voltage DC power supply and a reactor. As shown in Figure 2, the reactor which was a cylindrical reaction vessel with an inner diameter of 80 mm was used. The anode, from which the discharge was emitted, was a pointed platinum wire (id=0.5 mm) sealed into a quartz tube. The cathode was a graphite bar (id=10 mm). The reaction vessel was coated by a water jacket for circulating the cell with cooling water to keep the reaction system temperature constant. The solution for treatment was prepared by dissolving T-2 toxin or patulin in acetonitrile and diluted with 2 g/L sodium sulfate solution (pH=6.5). A 150 mL portion of the solution was poured into the reaction vessel for treatment. The pH of solution was adjusted with 0.1 mol/L NaOH or H_2SO_4 to an expected value. The DC high voltage of 540 V was applied across electrodes through a DC power source (variable voltage 0-1000 V and current of 0-1000 mA) to start the reaction. During the reaction, the solution was gently stirred through a magnetic stirrer and a small portion of solution was periodically sampled out for high performance liquid chromatography (HPLC, Angilent 1100) with UV detector analysis to follow the variation of T-2 toxin and patulin concentration and analyze its degraded products. The separation was performed using an Ultimate XB-C18 reversed-phase column at a flow rate of 1.0 mL/min. Identification and quantitative analysis of the intermediate products were based on the peak retention times and calibration with authentic standards. For T-2 toxin, mobile phase was aqueous solution containing 50% acetonitrile, products were detected at 254 nm while for patulin, mobile phase was aqueous solution containing 10% acetonitrile and products were detected at 276 nm. Apple fruits were supplied from a local market in Lanzhou, China. Apples were washed with water to remove surface dirt and were mashed in a Waring blender and manually pressed using double-layer cheesecloth to obtain raw apple juice. The juice was stored at 4°C until it was subsequently used (Figure 2).

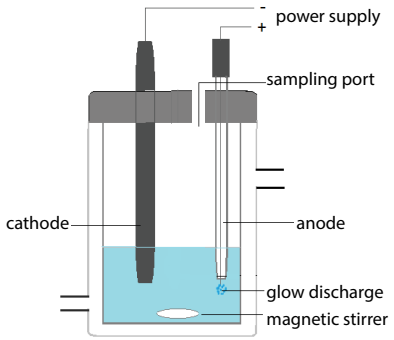

Figure 2: Schematic diagram of GDP reactor.

Results and Discussion

Variation of T-2 toxin and its degraded products concentration

Figure 3 shows the parts of HPLC chromatograms of samples from experiments. It shows the variation of T-2 toxin and its degraded products concentration during the treatment time. Figure 3a showed that the peak of T-2 toxin was high when the sample that was not degraded by GDP. Figure 3b indicated that after the sample was degraded 9 min by GDP, the peak of T-2 toxin reduced and some new peaks appeared. These new peaks were from the intermediate products of degradation of T-2 toxin. Figure 3c demonstrated that after 21 min, all the peaks of intermediate products reduced. When the treatment time reaches 40 min, the peaks of intermediate products and T-2 toxin all disappeared, this could be found in Figure 3d. These results indicate that T-2 toxin could be completely degraded by GDP.

Variation of solution pH value during degradation

In order to study the mechanism of degradation of T-2 toxin by GDP, the variation of pH value of the solution during treatment was investigated (Figure 4). It could be seen that the pH value reduced rapidly during the first 3 min and then it increased slowly from 10 to 30 min. These can be explained that when T-2 toxin was oxidized by ·OH radicals, a series of carboxylic acids were produced which caused the decrease of pH value initially. Then ·OH radicals would go further to oxidize the carboxylic acids producing the substances such as CO_2 and H_2O. This reaction resulted in high pH value.

Effect of initial concentration on degradation efficiency of T-2 toxin

Figure 5 shows the plot of degradation of T-2 toxin as a function of the reaction time at different initial concentrations of the substrate. As shown from the curve, the rate of elimination of T-2 toxin decreases with increasing concentrations of T-2 toxin in the starting solution. When the initial concentration of the reactants was 38 mg/L, 18% of the reactant amount was removed within 4 min. In the case of 13 mg/L, the removal rate was only 12%. This may be due to the utilization of hydroxyl radicals for degradation of T-2 toxin, which is the oxidant responsible for plasma oxidation and the coupling reaction of hydroxyl radicals. The higher the concentration of T-2 toxin, the higher utilization of the hydroxyl radicals would be. As a result, the rate of removal of T-2 toxin was higher at higher initial concentrations of the reactants within the same time period.

As the decay curves in Figure 3 appeared to be exponential, the date was fit to the integral rate equation for the first order reaction Eq. (1):

$$ln\,(C_0/C_t) = k\,t \quad (1)$$

Where C_0, C_t, k and t denote the initial concentration, the concentration at a given time, the apparent rate constant and the given time, respectively.

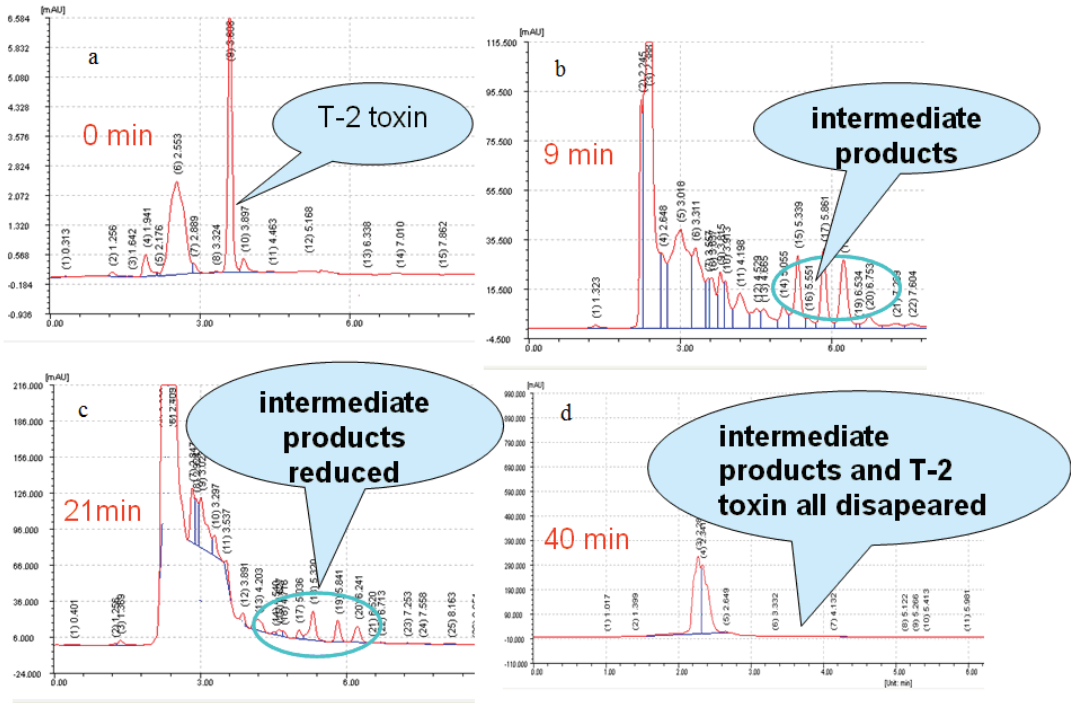

Figure 3: HPLC chromatograms of samples degraded by GDP after different time (a) not degraded; (b) degraded for 9 min; (c) degraded for 21 min; (d) degraded for 40 min.

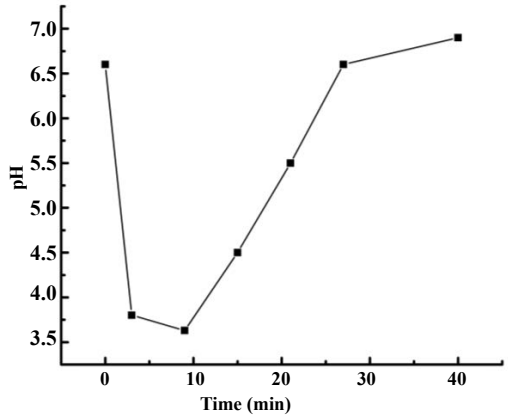

Figure 4: Variation of T-2 toxin solution pH value during treatment by GDP.

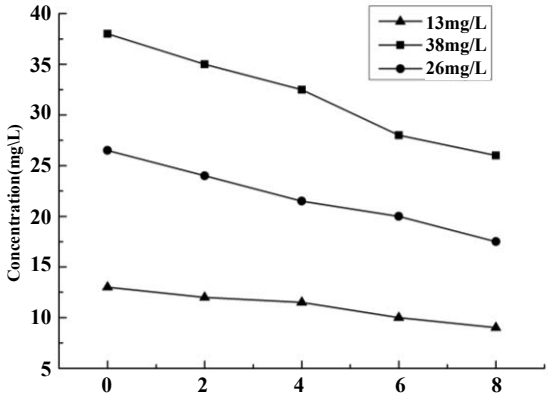

Figure 5: Effect of initial concentration on degradation efficiency of T-2 toxin.

For each set of date, a line with good correlation was obtained as shown in Figure 6. The results indicated that T-2 toxin would be depleted in a first-order manner.

The influence of pH on degradation efficiency of T-2 toxin

The pH of solution often plays an important role in the oxide degradation treatment. Figure 7 shows the effects of initial pH of the solution on the T-2 toxin removal. Faster removal was achieved in relatively higher acidity or alkalinity than in the neutral pH value. This could be attributed to the fact that the oxidative ability of the plasma was stronger in acidic conditions. The results agree with the observation that the oxidative potential of hydroxyl radical was 2.65 V at pH=4.0 while 2.50 V at pH=6.0 and in basic conditions the production is higher than in neutral condition [23].

Effect of catalysts on degradation efficiency of T-2 toxin

It is well known that some transition metal ions can catalyze the degradation of organic substrates in the advanced oxidative processes (AOP) when the oxidation reagents H_2O_2, O_2 and ClO_2 and so on were used. In the present study, the catalyses of Fe^{2+} and H_2O_2 were investigated (Figure 8). The value of pH was maintained at 4.0 since the catalytic activity of Fe^{2+} was found to be better in acidic conditions. The initial concentration of reactant was 38 mg/L. When the degradation reaction was catalyzed by Fe^{2+}, the elimination rate of the T-2 toxin after 8 min reached 60%. Also, when H_2O_2 was used as catalyst, 8 min later, the elimination rate of the T-2 toxin reached 50%.

It showed that Fe^{2+} has an evident catalytic effect on the degradation

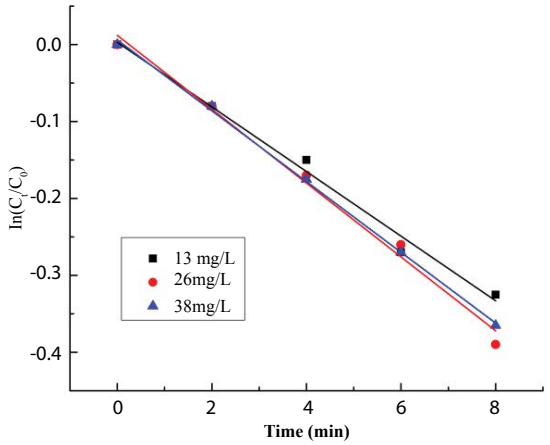

Figure 6: Lines fit to the integral rate equation.

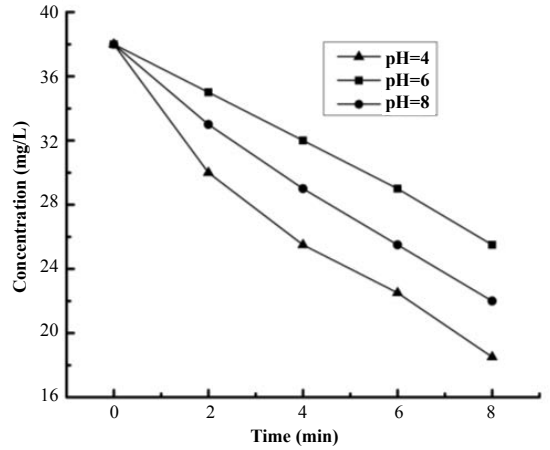

Figure 7: Effect of acidities on degradation efficiency of T-2 toxin.

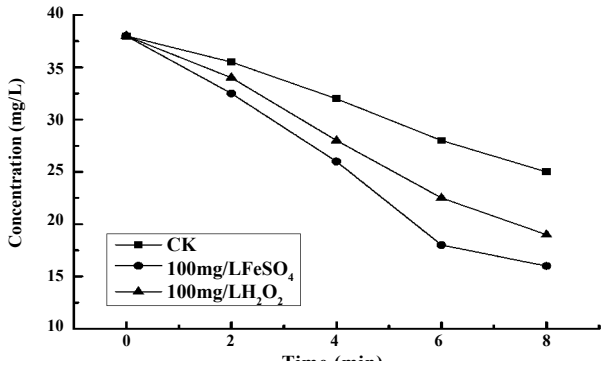

Figure 8: The catalyses of Fe^{2+} and H_2O_2.

of T-2 toxin by GDP under these experimental conditions, which is similar to the value obtained by other studies [28,30].

It also showed that H_2O_2 can accelerate the elimination speed of degradation of the T-2 toxin. The main cause is that addition of H_2O_2 increases the production of ·OH, which in turn accelerates the degradation reaction of T-2 toxin.

The changes of toxicity of T-2 toxin solution during GDP treatment

In order to investigate the change of toxicity of T-2 toxin during the degradation, the inhibition ability of T-2 toxin solution to germination of pea seeds was tested. There was no T-2 toxin in control sample. T-2 toxin solutions were treated by GDP for 0 min, 5 min, 10 min, 20 min and 30 min, respectively. Calculated germination rate of peas in these solutions increased after 24 h, 48 h and 72 h. The results as shown in Figures 9 and 10 suggested that germination rate of peas in degraded solution increased along with corresponding increase in degradation time. It explained that GDP could eliminate the toxicity of T-2 toxin.

Efficiency of degradation patulin in apple juice by GDP

Figure 11 shows the variation of patulin concentration in apple juice during the degradation time. It shows that when the initial concentration was 12 mg/L, the degradation rate reached 54% only in 3 min. 10 min later, more than 92% of patilin was degraded. There was no patulin detected when degradation time reached 35 min. These results indicate that patulin in apple juice could be also completely degraded by GDP.

Effect of GDP treatment on apple juice quality

In order to investigate the effect of GDP treatment on apple juice quality, the major quality parameters of apple juice of Fuji and Red Delicious apples were monitored during the study.

The results in Figures 12 and 13 showed that whether in Fuji apple juice or Red delicious apple juice, the soluble solid concentration, the total acid content, pH of apple juice had no remarkable changes within 10 min when treated by GDP while color value and clarity changed little.

The results from Tables 1 and 2 demonstrated that all of the viscosity, conductivity, turbidity and browning degree of Fuji apple juice and Red delicious apple juice had no remarkable change during treatment time by GDP.

Table 3 explained that when apple juice was treated by GDP for 10 min, the flavonoids and flavonols content remained constant, whilst only the total phenol was increased.

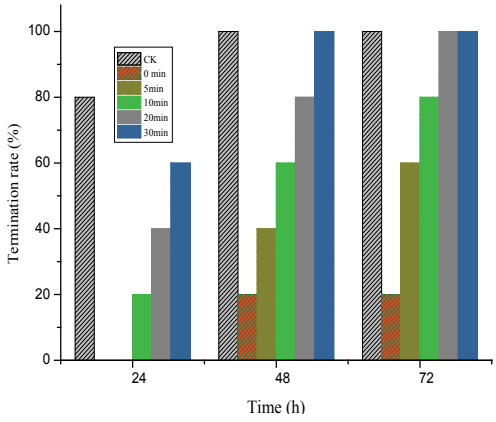

Figure 9: The germination rate of pea seeds in degraded solution.

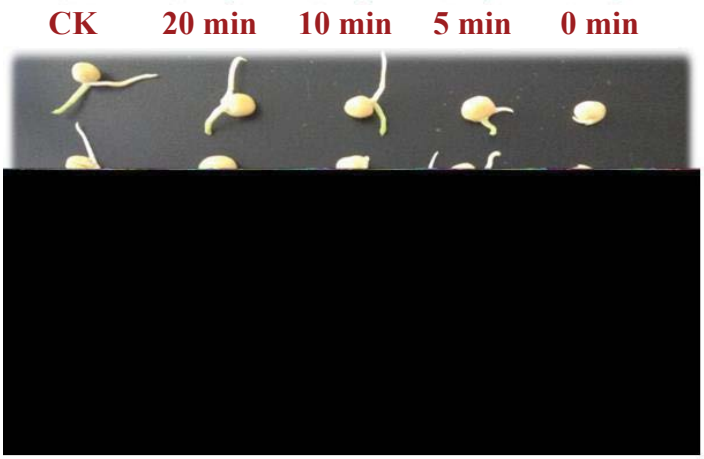

Figure 10: The photo of germination of pea seeds after 72 h.

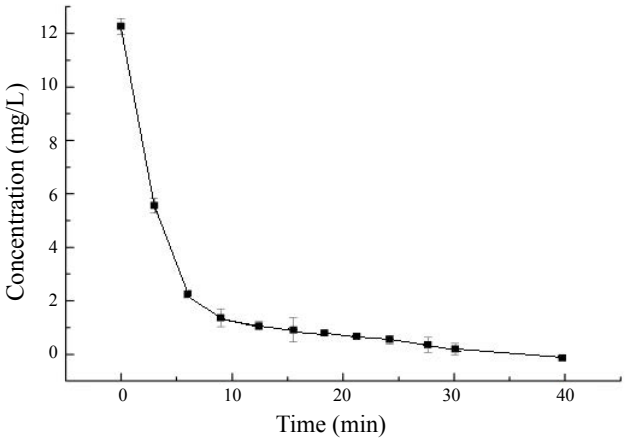

Figure 11: Variation of Patulin concentration in apple juice during treatment by GDP.

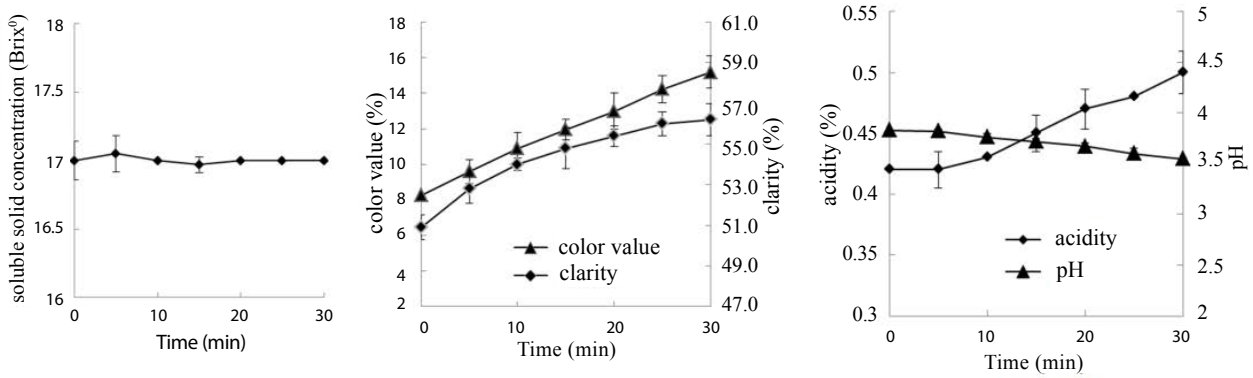

Figure 12: Effect of GDP on the soluble solid concentration, color value, clarity, acidity and pH of Fuji apple juice.

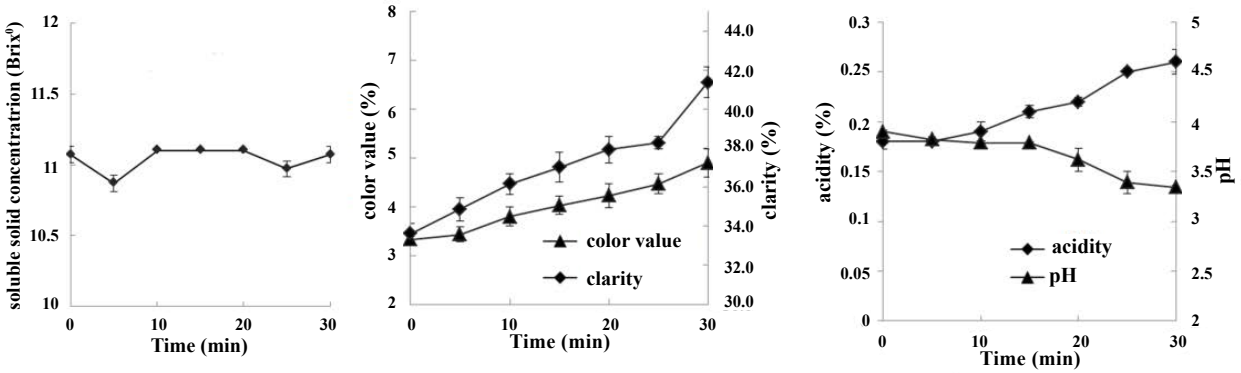

Figure 13: Effect of GDP on the soluble solid concentration, color value, clarity, acidity and pH of red delicious apple juice.

Treatment time (min)	Viscosity (Pa·s)	Conductivity (mS)	Turbidity	Browning degree
0	1.80 ± 0.000a	2.31 ± 0.006a	0.227 ± 0.002abc	1.248 ± 0.012a
5	1.80 ± 0.000a	2.31 ± 0.000a	0.209 ± 0.010ab	1.084 ± 0.056abc
10	1.80 ± 0.000a	2.34 ± 0.053a	0.203 ± 0.002a	1.001 ± 0.029b
15	1.81 ± 0.007a	2.49 ± 0.012b	0.217 ± 0.007abc	1.052 ± 0.031bc
20	1.81 ± 0.007a	2.49 ± 0.015b	0.224 ± 0.009abc	1.040 ± 0.107b
25	1.81 ± 0.014a	2.48 ± 0.006b	0.237 ± 0.008c	1.225 ± 0.040a
30	1.82 ± 0.007a	2.49 ± 0.000b	0.233 ± 0.030bc	1.205 ± 0.177ac

a, b, c: Differences between treatments, when Lowercase letters are completely different, mean Significant difference (P<0.05), otherwise, No significant difference (P>0.05)

Table 1: Effect of GDP on the viscosity, conductivity, turbidity and browning degree of Fuji apple juice.

Treatment time (min)	Viscosity (Pa·s)	Conductivity (mS)	Turbidity	Browning degree
0	1.80 ± 0.000a	2.22 ± 0.038a	0.415 ± 0.006abc	1.682 ± 0.004a
5	1.80 ± 0.028a	2.23 ± 0.031a	0.402 ± 0.006ab	1.698 ± 0.027a
10	1.80 ± 0.000a	2.23 ± 0.042a	0.383 ± 0.006ab	1.651 ± 0.035a
15	1.82 ± 0.049a	2.30 ± 0.015b	0.423 ± 0.025bc	1.726 ± 0.047a
20	1.79 ± 0.049a	2.31 ± 0.006b	0.453 ± 0.041c	1.656 ± 0.186a
25	1.80 ± 0.000a	2.40 ± 0.020c	0.371 ± 0.044a	1.630 ± 0.046a
30	1.81 ± 0.035a	2.43 ± 0.015c	0.381 ± 0.021ab	1.600 ± 0.084a

a, b, c: Differences between treatments, when Lowercase letters are completely different, mean Significant difference (P<0.05), otherwise, No significant difference (P>0.05)

Table 2: Effect of GDP on the viscosity, conductivity, turbidity and browning degree of red delicious apple juice.

Treatment time (min)	Flavonoids (μg/mL) (Fuji)	(Red Delicious)	Flavonols (μg/mL) (Fuji)	(Red Delicious)	Total phenol (μg/mL)
0	29.87 ± 0.15	39.11 ± 1.23	5.17 ± 0.06	7.63 ± 0.13	211.68 ± 1.27
5	29.98 ± 0.46	40.03 ± 0.54	5.18 ± 0.04	7.79 ± 0.19	425.15 ± 0.64
10	30.09 ± 1.08	40.68 ± 0.23	5.29 ± 0.02	7.83 ± 0.08	800.67 ± 0.58
15	37.26 ± 0.46	42.70 ± 0.92	5.81 ± 0.04	9.02 ± 0. 34	939.56 ± 1.20
20	52.64 ± 0.54	47.32 ± 0.23	8.52 ± 0.08	10.81 ± 0.13	1592.85 ± 0.92
25	62.04 ± 1.08	37.97 ± 0.85	9.78 ± 0.02	11.55 ± 0.29	1999.23 ± 2.04
30	73.73 ± 0.08	42.97 ± 0.08	12.01 ± 0.02	12.89 ± 0.08	2431.33 ± 2.65

Note: Results in above tables were given as mean ± SD of three independent determinations. One-way ANOVA was used to compare the means. Differences were considered to be significant at P<0.05.)

Table 3: Effect of GDP on the flavonoids, flavonols and total phenol content of apple juice.

Conclusion

Glow discharge plasma (GDP) provides many ·OH radicals in the system. T-2 toxin and patulin can be effectively and exhaustively degraded by GDP in aqueous solution or in apple juice, where the ·OH radicals are most responsible in the degradation of toxins. On the basis of detailed analysis of the intermediate and the changes of the pH value of solution during treatment, it is supposed that the oxidation degradation could be initiated by the hydroxylation of T-2 toxin or patulin. When the rings of T-2 toxin or patulin were broken, the major products were carboxylic acids. Eventually, all the acids were completely degraded into CO_2 and H_2O. So the final products were inorganic carbon and water. The Fe^{2+} and H_2O_2 can improve GDP to produce more ·OH radicals. The initial concentration of T-2 toxin and the pH value could also affect the degradation rate. The higher initial concentration, the higher treatment efficiency would be achieved.

The faster T-2 toxin removal rate was achieved at a relatively higher acidity and basify. GDP treatment for 10 min had no significant effects on major quality parameters of apple juice as the final products were inorganic carbon and water. GDP is an efficient and friendly method to degrade T-2 toxin in aqueous solution and patulin in apple juice.

Acknowledgement

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