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Effective degradation of the mycotoxin patulin in pear juice by porcine pancreatic lipase

Yanju Xiao (Master) (Co-author)[a,1], Bingjie Liu (Doctor) (Co-author)[a,1], Zijuan Wang (Master) (Co-author)[a], Chenlu Han (Master) (Co-author)[a], Xianghong Meng (Doctor) (Co-author)[a,b], Fang Zhang (Docter) (Corresponding author)[a,*]

[a] College of Food Science and Engineering, Ocean University of China, Qingdao, 266003, China
[b] Pilot National Laboratory for Marine Science and Technology, Qingdao, 266003, China

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ABSTRACT

Porcine pancreatic lipase (PPL) was used to degrade the mycotoxin patulin (PAT) in pear juice. The dosage of PPL, the initial concentration of PAT, reaction temperature and time were investigated by batch experiments to study the optimal degradation condition. The concentration of PAT in pear juice was determined by high performance liquid chromatography with an ultraviolet detector (HPLC-UV). The results showed that the optimal condition was 0.02 g PPL/mL pear juice at 40 °C for 24 h. The content of organic acids, volatile flavor components, polyphenols, ascorbic acid and the degree of browning reaction in pear juice, relating to the quality of juice, changed insignificantly. Although the initial PAT concentration was very high, the degradation product was confirmed nontoxic by cytotoxicity test of Caco-2 cells. It suggested that PPL could be further considered to be applied in the degradation of PAT in pear juice.

1. Introduction

The diet contaminated by mycotoxins is delivered to a large proportion of the worldwide population, especially in developing countries (Ortiz et al., 2018). Specifically, PAT (4-hydroxy-4H-furo [*3,2-c*] pyran-2 (*6H*)-one), classified as a mycotoxin and found initially in the 1940s, has a serious negative impact on the storage of food and fruit-processing industry (Sadok et al., 2018). This fungal secondary metabolite could be produced by more than 60 different species, particularly belonging to the genera *Penicillium, Aspergillus, Byssochlamys* (Tannous et al., 2016; Wright, 2015). It is well-known that PAT is a kind of enteropathogenic mycotoxins, it has been demonstrated by cells and animals' experiments. It was reported that PAT was rapidly absorbed and caused intestinal mucosal ulceration and inflammation by *in vitro* study (Speijers et al., 1988). The epithelium and immune cells of the intestinal mucosa are important targets for the toxic effects and PAT strongly inhibits the T cell proliferation (Assunção et al., 2016). Moreover, the transfer of PAT from fruits to corresponding product occur easily due to its solubility in water and stability under heat and acidic mediums (Raiola et al., 2012). European Commission Regulation recommends the allowable level of 50 μg PAT/kg fruit juices (European Commission, 2006) and the upper limit of PAT is more stringent for infants.

Various physical, chemical and biological approaches have been investigated to control PAT contamination in food and fruit juices (Zheng et al., 2018). Ozone detoxification and high hydrostatic pressure processing have been explored to reduce PAT in fruit juices (Diao et al., 2018; Hao et al., 2016). The main alternative materials studies include cross-linked xanthated chitosan resin (Peng et al., 2016), cysteine-functionalized metal-organic framework (Liu et al., 2019), boric acid (Lai et al., 2016) and other chemical additives such as sulphur compounds, ascorbic acid, thiamine hydrochloride, pyridoxine hydrochloride and calcium pantothenate (Ibarz et al., 2017). Many microorganisms displayed the ability to degrade or bio-transform PAT. For example, up to 80% of PAT was bio-transformed to *E*- and *Z*-ascladiol *in vitro* by *Lactobacillus plantarum*, and the use of lactic acid bacteria as biocontrol agents is potentially a promising method of increasing food quality and safety in a range of commodities (Hawar et al., 2013). Yeast, such as *Rhodosporidium paludigenum*, was also studied to transform PAT into desoxypatulinic acid, significantly less toxic to

* Corresponding author.
E-mail addresses: xiaoyan111308@163.com (Y. Xiao), liubj@ouc.edu.cn (B. Liu), 529974299@qq.com (Z. Wang), 2371516566@qq.com (C. Han), mengxh@ouc.edu.cn (X. Meng), zhangfang@ouc.edu.cn (F. Zhang).
[1] These authors contributed equally to this work.

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Arabidopsis thaliana and human liver cells (Zhu et al., 2015). It was intracellular enzyme of yeast *Rhodotorula mucilaginosa* that it could degrade PAT into deso-xypatulinic acid (Li et al., 2019). Besides, 1.0 kGy γ-irradiation has been used to degrade PAT, and only 2.3% of PAT was remained in the 1% citric acid aqueous solution, but the nutrient elements should be considered because the radio-degradation effects are environment dependent (Yun et al., 2008). Another new biosorbent of magnetic Fe_3O_4@chitosan nanoparticles coated with inactivated bacteria strains was studied for the removal of PAT, over 90% of PAT was reduced and there was not any significant negative impact on the quality parameters of orange juice (Ge et al., 2017). Nevertheless, current strategies were not entirely available because of high expenses, chemical hazard during the manufacturing process, environmental influence, or practical difficulties involved (Tang et al., 2018). In general, reliable and effective methods to degrade PAT in different products were worth exploring for reducing its damage to consumers.

Porcine pancreatic lipase (PPL), identified possessing 50–52 kDa molecular weight of tiny globulin form, assembles a single chain of 449 amino acids. The globulin contains six disulfide bridges and two free thiol groups, the catalytic triad is composed of serine (Ser), histidine (His) and aspartate (Asp). The study showed that PPL executed better overall hydrolysis rate in organic-aqueous mixture solvent systems (Shen et al., 2008). The mechanism of lipase catalysis is similar to serine protease catalysis with the formation of two tetrahedral intermediates, and it involves nucleophilic attack of hydroxyl terminal serine residues (presented in active sites) against the ester bonds of sensitive substrate (Tang et al., 2018). It is one of the most extensive lipases used for biotransformation or chemical reactions due to its accessibility, high stability and broad specificity. Meanwhile, PPL is the cheapest lipase commercially available in different sources (animal, plant or microbial cells). (Mendes et al., 2012). As one of the most economically important fruit crops, pears are cultivated in more than 50 countries or regions and consumed all over the world due to their high nutrition and desirable taste (Zhou et al., 2018). However, there are usually rotten pears infected by *P. expansum* and PAT cannot be prevented just by removing the rotten part, so it enters the human food supply (Wei et al., 2017).

The aim of this work was to study the potential of PPL to degrade PAT in pear juice and the impact on the quality parameters of pear juice. Batch degradation experiments, including different PPL dosages, PAT concentration, temperature and time, were performed to improve the degree of degradation. Ingredients of pear juice, such as organic acids, vitamin C, polyphenols and volatile flavor compound that would be affected by hydrolytic activity of PPL, were determined. Finally, the test involving CaCo-2 cells was conducted to assess the toxicity of degradation product.

2. Materials and methods

2.1. Materials

Pure pear juice (100% juice content, Brix 70.0, pH 4.3–4.5) was obtained from local supermarket (Qingdao, China). PAT standard solution (100 mg/L) and acetonitrile of chromatographic purity were obtained from Sigma-Aldich Chemical Co., Ltd (St. Louis, MO, USA). Porcine pancreatic lipase (PPL, Type II, EC 3.1.1.3, biological reagent, 30 U/mg) and standard agents of malic acid, citric acid, 3, 4, 5-trihydroxybenzoic acid were supplied by Yuanye Biotechnology Co., Ltd (Shanghai, China). Dimethyl sulfoxide (DMSO) and 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) were supplied by Solarbio Science and Technology Co., Ltd (Beijing, China). Other reagents were of analytical purity and deionized water was used for all of the experiments.

2.2. Preparation of PAT solution

PAT standard solution (100 mg/L), stored at −20 °C, was dissolved in acetic aqueous solution (pH 4.0) to obtain 10 mg/L solution for test, preserved in the dark at 4 °C before used. Different concentration of PAT samples was prepared using the same solution.

2.3. Quantification of PAT by HPLC-UV

PAT, before and after degraded by PPL in pear juice, was extracted by 2 mL ethyl acetate for three times, stated on a shaker-incubator at 180 r/min for 1 h, then centrifuged at 4000 *g* for 1 min at 4 °C. Three supernatants of a sample were mixed together and the solvent was immediately evaporated to dryness under a nitrogen stream and reconstituted in 1 mL acetic acid aqueous solution for detection.

Quantitative detection of PAT was determined by HPLC equipped with an UV detector. The HPLC apparatus own a 1260 analytical system with an Agilent ZORBAX SB-C18 analytical column, 5 μm × 4.6 mm × 250 mm (Agilent Technologies Co., Ltd., Santa Clara, CA, USA). The mobile phase was an equilibrium mixture of acetonitrile/deionized distilled water (1:9, v/v), with a flow rate of 1 mL/min at 30 °C. Each sample with 20 μL was injected into the column and chromatogram for calculation was extracted at 276 nm. All reagents and samples were filtered by 0.45 μm pore size filters before loaded. The HPLC column was handled by running a background without injection. The requirements for the recovery of these samples were set to 60–115% (Liu et al., 2015). The limit of detection and quantification were 1.5 and 3.5 μg/L in pear juice, respectively. The degree of PAT degradation (μ) by PPL was calculated by the following equation:

$$\mu = \frac{P_0 - P_f}{P_0} \times 100\% \quad (1)$$

where, μ is degradation degree (%), P_0 and P_f are the peak areas (retention time 5.9 min) of PAT in pear juice before and after degradation.

2.4. Batch degradation experiments

Batch degradation experiments of PAT by PPL in pear juice were performed to assess enzymatic hydrolysis efficiency. One milliliter of pear juice was fully mixed with 1 mL PAT solution of 0.75 mg/L and PPL of 0.04 g in a medium gland reaction tube, which was then stated on a shaker-incubator at 180 r/min, 25 °C for 24 h. The control was prepared with the absence of PPL in pear juice. Firstly, the effect of PPL amount of 0.005, 0.01, 0.015, 0.02, 0.025, 0.03 g/mL (corresponding to 1.5–9.0 × 100 U/mL) on PAT degradation was determined. The effect of initial PAT concentration on degradation was tested from 0.125 to 1.0 mg/L, with a gradient of 0.125 mg/L. Then, the effect of reaction temperature was conducted at 15–45 °C. Finally, the effect of reaction time on degradation was studied for 6–42 h, with a gradient of 6 h. All tests were studied under a nature pH 4.2–4.5 of 100% pear juice and a range of pH 4.0–5.0 prompted a relevant higher activity of PPL (Li et al., 2017).

2.5. Determination of organic acids, flavor compounds, vitamin C, polyphenols and browning index

These characterizations for evaluating the impact of PAT degradation to pear juice were chosen on the basis of Tang et al. (2018). They could be affected by enzymatic hydrolysis of PPL, which further affected the quality of pear juice.

The determination of organic acids was performed according to the method reported by Oliveira et al. (2008) with slight adjustments. Samples to be tested by HPLC-UV were treated by 0.45 μm pore size filter before analysis. Organic acids were separated on a column of Agilent ZORBAX SB-C18, 5 μm × 4.6 mm × 250 mm (Agilent Technologies Co., Ltd., Santa Clara, CA, USA) at 30 °C, with 20 μL of sample

injected. The mixture of 0.01 mol/L phosphate buffer and acetonitrile (19:1, v/v) was applied as the mobile phase, with a flow rate of 0.5 mL/min and detection at 210 nm.

The volatile flavor compounds were determined by GC/MS with solid phase microextraction (SPME) as previously reported in the literature (Miyawaki et al., 2016). 10 mL of a sample and 2.5 g NaCl were fully mixed and equilibrated in a water bath of 40 °C held for 5 min. Next, the SPME fiber (50/30 μm, DVB/CAR/PDMS (Grey), Supelco Inc., Bellefonte, PA, USA) was inserted into the headspace of a vial for 40 min to extract and adsorb flavor components. Then, it was removed from the vial and inserted into the injection port of GC/MS (6890 N/5973, Agilent Technologies Co., Ltd., Santa Clara, CA, USA) with Agilent HP-5 (25 m × 0.32 mm × 0.17 μm), employing helium as carrier gas at a flow rate of 1.0 mL/min. The oven temperature, controlled by a program, was raised to 250 °C (held for 6 min) from initial 40 °C (held for 2.5 min) at a rate of 6 °C/min. The mass spectra were obtained by electron-impact ionization at an ionization voltage of 70 eV, with a scanning range of 35–450 amu. The temperature of the transmission line and the inlet were 230 and 250 °C, while the ion source and mass spectrometer interface temperatures were 250 and 280 °C, respectively. NIST02 mass spectra database was employed to ascertain GC peaks.

Vitamin C owning strong reducibility could be oxidized by Iodine quantitatively and determined via titrimetric method (Sapei and Hwa, 2014). A sample solution of 20 mL was mixed with 75 mL distilled water and 5 mL acetic acid in 250 mL Iodine volumetric flasks. The aqueous solution of samples (pear juice before and after PAT degradation) and control (an acetic acid aqueous solution) were both titrated with iodine standard using amidin as an indicator. The content of vitamin C was calculated according to the consumed volume of the iodine standard titration solution. The vitamin C content (W) was calculated by the following equation:

$$W = \frac{c \times (V_b - V_a) \times M}{V} \times 100 \quad (2)$$

where, W is the concentration of vitamin C (mg/100 mL), V_b is the volume of iodine standard consumed by sample (mL), V_a is consumed by control (mL), c represents the concentration of iodine standard solution (0.05 mol/L), M (g/mol) is the molar mass of vitamin C that is equal to 176.12, V is the volume of sample (mL).

The polyphenols in pear juice were determined by the tartaric acid colorimetric method at the wavelength of 540 nm (Tang et al., 2018). Standard solution of 3, 4, 5-trihydroxybenzoic acid (1 mg/mL) of 0.25–1.5 mL was added into 25 mL colorimetric cylinder, mixed with 5 mL ferrous tartrate solution and deionized distilled water of certain volume to reach 10 mL compounds. Subsequently, all mixtures were diluted with phosphate buffer (pH 7.5) to the tick mark of 25 mL. Control groups of deionized distilled water were performed the same procedure as the treatment groups. The content of polyphenols was calculated based on the calibration curve, which was expressed as the correlation of absorbance and the content of 3, 4, 5-trihydroxybenzoic acid. The amount of 3, 4, 5-trihydroxybenzoic acid (mg) was the abscissa; an absorbance value at 540 nm was the vertical coordinate.

Colorimetric method was also chosen to validate the browning degree during enzymatic hydrolysis of PAT. Samples were filtered by 30–50 μm filter paper and the variety absorbance of filtrates was measured through a WFJ2100 spectrophotometer (Unico Co., Ltd, Shanghai, China). The degree of browning reaction was indicated by the absorbance value at the wavelength of 420 nm.

2.6. Cell viability analysis of PAT degradation product

Caco-2 cells, derived from a human colorectal adenocarcinoma and possessing similar structure and function with differentiated intestinal epithelial cells, were purchased from European Collection of Animal Cell Cultures (ECACC, number 88081201, Salisbury, UK) for MTT assay of PAT degradation product. Cells were cultured at 37 °C and 5% (v/v) CO_2 in Dulbecco's modified Eagle medium (DMEM). Detoxifications of 1.0, 5.0, 10.0 mg/L PAT were implemented and the product was extracted by the method mentioned above. Afterward, Caco-2 cell monolayer was cultivated for 24 h in 96-well plate until suitable density for tests and then cultivated with control (PBS), non-degraded PAT (1.0, 5.0, 10.0 mg/L) and degradation product of PAT by PPL in different concentration for 24 h. After co-cultured with MTT for another 4 h, 150 μL DMSO was poured slowly in the well for the microplate reader at 570 nm by an MQX200R2 BioTek Instruments (BioTek Instrument Co., Ltd, Winooski, Vermont, USA).

2.7. Statistical analysis

All experiments were conducted in triplicate and the statistical analyses of the data were evaluated using a one-way analysis of variance (ANOVA), and Turkey's tests were performed to identify significant differences ($p < 0.05$). All the data were reported as means ± standard deviation.

3. Results and discussion

3.1. Effect of PPL concentration, initial PAT concentration, reaction temperature and time on PAT degradation

The effect of PPL concentration on the degradation degree of pear juice can be seen in Fig. 1A. The results showed that PAT degradation rate enhanced obviously with increasing PPL amount (0.005–0.02 g/mL). Later, it slowed down and had no significant difference though further growth of PPL concentration and 42% of PAT (0.75 mg/L) in pear juice was degraded by depleting less PPL (0.02 g/mL). Primely, lower PPL amount could not satisfy the need of PAT degradation, so the degree of PAT degradation enhanced with increasing PPL amount. With degradation of PAT by more PPL, limited medium volume may arise opposite effects on hydrolysis efficiency as displayed in dietary polysaccharides and there would be a feedback inhibition mechanism (Zhang et al., 2019). That is, the degradation efficiency cannot enhance indefinitely with the increase of PPL quantity.

The concentration of PAT in diverse samples was different. To measure the detoxification capability of PPL, varied PAT concentration for degradation was investigated. As shown in Fig. 1B, PAT degradation could achieve 93.4% at concentration of 0.125 mg/L. The degradation rate was 31.26% for 2.0 mg/L of PAT. It was reported that the conformational changes around the lid (some elements of secondary structure) controlled the PPL activity and the open conformation allowed substrates to reach the active site residues (Haque and Prabhu, 2016). A certain amount of PPL might not expose enough active site for PAT degradation (Hao et al., 2016) and PAT pollution in pear juice should be controlled to reduce public health risks.

The impact of temperature on PAT degradation was evaluated from 15 to 45 °C and the results were demonstrated in Fig. 1C. The maximal degradation rate of 61.38% was measured and significantly higher than that of other temperature. In this study, PPL owned the maximal activity at 40 °C. Generally, PPL is a mesophilic enzyme, having the maximal activity in the range of 35–45 °C (Mendes et al., 2012). The effect of time on PAT detoxification was illustrated from Fig. 1D. The optimum time for degradation was within 24 h, and there was no growth by extending time because the inactivation of PPL virtually. The kinetics of PAT degradation in pear juice followed diphasic behavior with an initial rapid degradation and followed by a slow rate at the extended treatment time (Hao et al., 2016). As reported, the lipase was practically inactivated after 2 days of incubation in buffer solution at 35 °C (Desai et al., 2006). Therefore, it was demonstrated that the PPL activity was reduced gradually with extending degradation time. In the study, the optimal dosage of PPL was 0.02 g/mL at 40 °C within 24 h when the PAT concentration was 0.375 mg/L. Although the concentration was still more than twice of the limit, the degradation degree

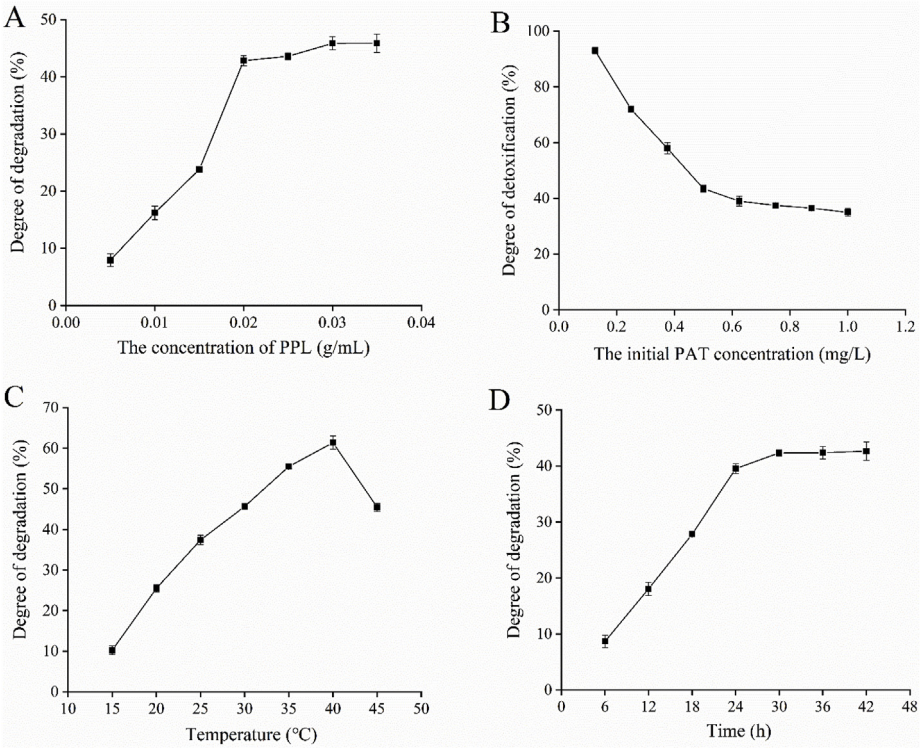

Fig. 1. Effect of PPL concentration (a), initial PAT concentration (b), reaction temperature (c) and time (d) on PAT degradation degree. The degree of degradation varied in parameters. All tests were performed in triplicates and the bars represented the standard deviations.

could exceed 93% as PAT concentration was lowered to 0.125 mg/L. That is, the mycotoxin PAT must be controlled before its contamination was expanded.

3.2. Effects of PAT degradation on organic acids, flavor compounds, vitamin C, polyphenols and browning degree

Organic acids are widely distributed in fruit juices and merit study due to their important role as natural antimicrobial agents and flavor enhancement. The nature and concentration of organic acids are both important factors influencing the organoleptic characteristics of fruit and vegetables, namely their flavor (Nawirska-Olszańska et al., 2014). Organic acids present in fruit, mainly malic and citric acid, exert an alkalizing effect in the human body, inhibit the growth of undesirable microflora, as well as exert influence on the course of metabolic processes and a mean to evaluate food processing (Oliveira et al., 2008). The content of organic acids in pear juice was depicted in Fig. 2. Indeed, malic acid and citric acid were the main acid and the peak time of their standards was 5.76 min and 7.41 min, respectively. The contents of malic acid and citric acid were 1.96/2.15 g/L and 302/308 mg/L before and after degradation. The contents of malic and citric acid increased by 10.0% and 2.1%, respectively, due to the hydrolysis of esters.

The influence of the enzymatic hydrolysis on the flavor components was shown in Table 1. The characteristic volatiles were esters, aldehydes, alcohols, and ketones (Liu et al., 2018b). Eleven kinds of compounds were identified and all were well-known odorants contributing to the flavor (Mendes et al., 2012). Obvious changes of flavor compounds were not observed after PAT degradation. As seen in Table 1, most of the ingredients in pear juice reduced insignificantly and 5-isopropyl-2-methylphenol was not detected. On the contrary, ethanol and ethyl acetate increased marginally. As versatile biocatalysts, lipases can catalyze esterification, interesterification, acidolysis, alcoholysis and aminolysis (Canada, 2004). Therefore, the application of PPL would be responsible for slight changes of flavor components in pear juice.

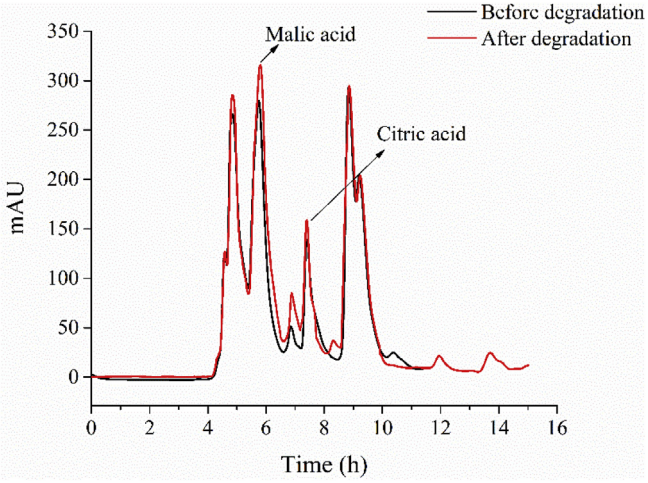

Fig. 2. Effect of PAT degradation on organic acids. The degradation of PAT (0.375 mg/l) in pear juice by 0.02 g/ml of PPL at 40 °C for 24 h. Chromatogram of pear juice by HPLC-UV before and after PAT degradation at 210 nm. The peak time of malic acid and citric acid standards are 5.76 min and 7.41 min, respectively.

Known as ascorbic acid, vitamin C is one of the beneficial ingredients in most fruit and vegetable juices and it is decomposed easily (Sapei and Hwa, 2014). In this experiment, the change of vitamin C was listed in Table 2. The content of vitamin C was 22.98 mg/100 mL and decreased to 22.67 mg/100 mL after enzymolysis procedure, which might be caused by exposure to light and incomplete elimination of oxygen. The singlet oxygen generated by a metal-catalyzed oxidation of ascorbic acid could attack the conjugated double bonds of PAT in specific matrix (Brackett and Marth, 1979). Presumably, vitamin C was barely oxidized and hydrolyzed by PPL in pear juice during the PAT degradation process.

Table 1
The relative content of flavor substances in pear juice before and after enzymatic reaction.

Numbers	Flavor components	The content (%)	
		Before degradation	After degradation
1	Ethanol	3.31 ± 0.02[a]	3.72 ± 0.03[a]
2	Ethyl acetate	9.53 ± 0.15[a]	9.67 ± 0.24[a]
3	Ethyl propionate	18.57 ± 0.33[a]	17.61 ± 0.18[a]
4	Butyl butyrate	12.37 ± 0.13[a]	11.01 ± 0.25[a]
5	Isopropanol	7.54 ± 0.11[a]	6.90 ± 0.04[a]
6	Acetaldehyde	0.51 ± 0.03[a]	0.44 ± 0.01[a]
7	Butyraldehyde	3.48 ± 0.06[a]	3.05 ± 0.16[a]
8	Furfural	0.77 ± 0.05[a]	0.64 ± 0.02[a]
9	5-isopropyl-2-methylphenol	0.15 ± 0.02[a]	Not detected[b]
10	7-methyl-1,6-octadiene	0.91 ± 0.04[a]	0.87 ± 0.05[a]
11	2-nonanone	21.01 ± 1.03[a]	18.07 ± 0.47[b]

The detection of flavor compounds in pear juice before and after the PAT (0.375 mg/L) degraded by PPL (0.02 g/mL) in triplicates. The results are expressed as the mean ± standard deviation. Data in the same line with different lowercase letters are significantly different ($p < 0.05$).

Table 2
Effect of PAT degradation on vitamin C, polyphenols, and the browning degree in pear juice.

	Vitamin C (mg/100 mL)	Polyphenols (mg/mL)	Browning degree
Before degradation	22.98 ± 0.17[a]	0.13 ± 0.01[a]	0.48 ± 0.04[a]
After degradation	22.67 ± 0.23[a]	0.12 ± 0.01[a]	0.49 ± 0.01[a]

The determinations of vitamin C, polyphenols, and the browning degree in pear juice before and after PAT (0.375 mg/L) detoxified by PPL (0.02 g/mL) in triplicates. The results are expressed as the mean ± standard deviation. Data with different lowercase letters are significantly different ($p < 0.05$).

Polyphenols, a crucial group of secondary metabolites extensively distributed in plants, possess strong natural antioxidants linking to health benefits. It can interact with certain proteins by primarily hydrophobic interactions and hydrogen bonds (Adrar et al., 2019). The calibration curve of 3, 4, 5-trihydroxybenzoic acid was expressed as $y = 0.6016x + 0.0074$ ($R^2 = 0.9999$). Also, the content of polyphenols was reduced from 0.129 to 0.122 mg/mL in Table 2, without significant change. It indicated that there were no apparent interactions between polyphenols and PPL. That is, the antioxidant activity of polyphenols in pear juice was well preserved after PPL enzymatic hydrolysis. Finally, browning often occurs in the processing of fruits and juices, which affects the color and flavor of the product. In our study, the browning degree of pear juice was presented by absorbance. It was 0.478 and 0.486 before and after the enzymatic hydrolysis, respectively, which changed insignificantly. It was declared that the browning reaction can be caused by polyphenol oxidase and inhibited by L-cysteine (Zhou et al., 2018). In the case, the enzymolysis procedure did not substantially affect the color perception of pear juice and limited the browning reaction.

3.3. Cytotoxicity in Caco-2 cells

The degradation product of PAT has been mainly reported as ascladiol and desoxypatulinic acid. Ascladiol and another unknown final product exhibited no effect on cell proliferation by Het-1a cell through cell viability analysis (Zheng et al., 2018). Meanwhile, ascladiol had also been verified apparently non-toxic to porcine intestinal tissues (Maidana et al., 2016). It was inferred that PAT was degraded with ring opening reaction by PPL in aqueous acetic acid solution, and the degradation product was desoxypatulinic acid (Molecular weight 159.06 for $C_7H_{11}O_4^+$) in our previous research (Liu et al., 2018a). However, there were little literatures on the toxicity of degradation products desoxypatulinic acid. Our results of cytotoxicity experiments of PAT degradation product by PPL in Caco-2 cells was illustrated in Fig. 3. The cell viability was up to 96% when they were infected with degraded PAT (1.0 mg/L). All the three groups with different PAT concentration promoted the cell survivability by over 40%. In short, it was demonstrated that the degradation product of PAT by PPL in our study was considerably less toxic to intestinal cells even though the initial PAT concentration well above the standard level.

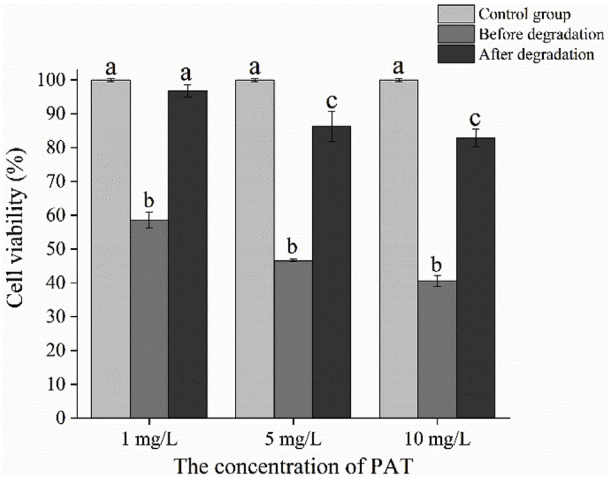

Fig. 3. The result of cytotoxicity test. The viability of Caco-2 cells (cultured in 96-well plate) treated with PBS, PAT (1, 5, 10 mg/l) before and after degraded by PPL. The cell viability of control group (PBS) was set as 100%, which the cell viabilities of groups before and after PAT degradation were calculated based on. Data in columns with different lowercase letters are significantly different ($p < 0.05$).

4. Conclusions

In summary, PPL was selected for the degradation of PAT in pear juice. The degree of PAT degradation was strongly dependent on the dosage of PPL, initial PAT concentration, degradation temperature and time. The desired condition of detoxification was 0.02 g/mL PPL to 0.375 mg/L PAT at 40 °C within 24 h. Additionally, the content of organic acids, aroma compounds, vitamin C, polyphenols, browning degree changed insignificantly. It declared that the quality and nutrition of pear juice were not dropped with the effective degradation of PAT by PPL. Besides, the degradation product of PAT was confirmed much less toxic or without toxic considerably by cytotoxicity test in Caco-2 cells, even at quite high concentration. Based on these facts, PPL was an interesting alternative material to detoxify PAT in pear juice.

Conflicts of interest

The authors report no conflict of interest.

Acknowledgments

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