Effects of ozone processing on patulin, phenolic compounds and organic acids in apple juice

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Abstract In this study, ozone processing was used to degrade patulin in apple juice, and the ozonolysis efficiency of patulin and its effects on phenolic compounds and organic acids in apple juice were investigated. Ozone processing was performed using a self-developed ozonolysis reactor at the ozone concentration of 12 mg/L and flow rate of 3 L/min for increasing ozonation times ranged from 0 to 30 min. Ozone processing significantly degraded patulin in apple juice, and decreased it from 201.06 to below 50 µg/L within 15 min, with a reduction of 75.36%. While major phenolic compounds and organic acids in apple juice were seriously destroyed by ozone processing compared with the control. Processors should consider the adverse effects of ozone processing on quality of apple juices and further studies are advised to optimize the ozone processing for remaining the phenols and organic acids in apple juices.

Keywords Apple juice · Patulin · Ozone processing · Phenolic compounds · Organic acids

Introduction

Patulin is a polyketide lactone (Fig. 1) mainly produced by *Penicillium expansum*, which commonly contaminates apples and their products all over the world (Beretta et al. 2000). Patulin can be easily dissolved in water and is very stable to heat in acidic condition, so it is often found in apple juice and difficult to be removed by heat processing. Many experiments have verified that patulin has the mutagenic, genotoxic, immunotoxic, teratogenic and cytotoxic effects (Puel et al. 2010). Therefore, some organizations established the maximum permitted level of 50 µg/L in apple juice (WHO 1995).

Based on the adverse effects of patulin on the human health and quality of agricultural products, people have been looking for ideal methods to degrade or remove it from contaminated foods. Generally, ideal detoxification methods must include: (1) inactive, destroy, or remove the toxin, (2) not produce or leave new toxic substances, (3) retain nutritive value/acceptability of product, (4) not significantly alter the processing technology of product, and (5) if possible, destroy fungal spores, (6) be practical in so far as it is technologically and economically feasible (Moake et al. 2005). Presently, the main methods for removing patulin in juices are clarification or adsorption treatments (Diao et al. 2018a). However, the clarification techniques varied significantly in their efficiencies for the removal of patulin in juices with different adsorbents (Gökmen et al. 2001). Additionally, these techniques also caused significant decreases in phenols (Kammerer et al. 2007). Therefore, people have been exploring novel and better techniques for degrading or removing patulin in juices, and ozone processing (OP) is the main one of them (Diao et al. 2018b). Ozone is a strong oxidant and potent disinfecting agent, which has been widely applied in

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Fig. 1 Chemical structure of patulin

treating water, fruits and vegetables for sterilization and extending their shelf-life with two forms, i.e. gaseous ozone and ozonized water (Miller et al. 2013). So OP is technologically applicable in food processing and economically feasible in ozone production. Specially, ozone can rapidly decompose to oxygen without toxic residues, making it environmentally friendly (Miller et al. 2013). Since 1997, McKenzie et al. found the degradation of mycotoxins by ozone (including patulin), some researchers began to study its application in degrading patulin in model system (Cataldo 2008; Ashirif-Gogofio et al. 2009). They concluded that ozone could effectively degrade patulin in water or model system, and deduced that the ozonolysis of patulin was due to the presence of a polyketide lactone on its structure, which made it highly vulnerable to oxidation (Ashirif-Gogofio et al. 2009). Therefore, OP can meet almost all the ideal detoxification conditions mentioned above.

Meanwhile, many literatures have also reported that OP could significantly affect the quality of foods (Tiwari et al. 2009; Torres et al. 2011). Especially for the phenolic compounds and organic acids in apple juice, they mainly contribute to the color and flavor of juices (Chinnici et al. 2005; Tiwari et al. 2008). Various processing can destroy them and affect the color and flavor of juices due to their highly sensitive to heat, oxygen, light, metal ions, adsorbents (Tiwari et al. 2008; Torres et al. 2011). Ozone, as a strong oxidant (oxidation potential + 2.07 eV), is also expected to affect phenolic compounds and organic acids in apple juices. And the color and flavor of apple juices are especially concerned by consumers and processors, which decide the juice quality and consumer purchase.

However, most of the detoxification experiments mentioned above were carried out in water or model system, which could not show the real degradation efficiency of patulin in fresh apple juice using an ozonolysis reactor for continuous detoxification. And the losses of phenolic compounds and organic acids in apple juices during ozone processing were still unknown. Hence, the objective of this study was to explore the degradation efficiency of patulin in fresh apple juices using an ozonolysis reactor, and

investigate the effects of OP on the phenolic compounds and organic acids in the ozonized apple juices.

Materials and methods

Materials

The standard patulin (purity $w > 0.98$) and Folin–Ciocalteu reagent were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The phenolic standards (gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acids, *o*-coumaric acids, ferulic acid, quercetin, catechin, and epicatechin, purity $w \geq 0.995$) and organic acid standards (malic acid, acetic acid, shikimic acid, succinic acid, maleic acid, ascorbic acid, purity $w \geq 0.995$) commonly present in apple juices were all obtained from ZIQIBIO Co. LTD. (Shanghai, China).

Preparation of fresh apple juices

Apples used in this study were the variety of Red Fuji, which were harvested in the fall of 2016 from Yantai city in China. Apples were pressed into juices using a juice extractor (Model SJ-F10S, SENUO, Guangdong, China). The clear apple juices were obtained by removing the juice pulp with a double layer of cheesecloth. The standard solution of patulin (1.25 mg/mL in deionized water) was added into the clear apple juices and the final patulin concentration was about 200 $\mu\text{g/L}$ ($201.06 \pm 2.70 \mu\text{g/L}$). All samples were placed in 1000 mL of opaque plastic bottles, and stored at -20°C prior to ozone detoxification. Frozen juices were used within 1 month of juice preparation.

Ozone processing equipment and its application

An ozone processing equipment used to degrade patulin in apple juice is shown in Fig. 2. It includes an ozone generator (Model DJ-Q2020A, Jinhua city, China; ozone is produced by electrolyzing water at low pressure), gas dryer (to remove water from ozone gas with anhydrous calcium chloride), ozone analyzer (Model IDEAL-2000, Zibo city, China), gas flow-meter (Model LZB-4, Yancheng city, China; to control the flow rate of ozone), ozonolysis reactor (Patent No. 201720358832.X, to degrade patulin in apple juice), and ozone destructor (Patent No. 201820102672.7, to decompose untapped ozone to oxygen).

Throughout the experiments, ozone concentration and its flow rate were 12 mg/L and 3 L/min, respectively, which were used to treat 200 mL of apple juice each time for ozonation times ranged from 0 to 30 min with an interval of 5 min at room temperature.

Fig. 2 Ozone processing equipment self-developed for degrading patulin in apple juices

Patulin content

Determination of patulin was performed using HPLC method reported by Li et al. (2005) with little modification. 10 mL of sample was added to 60 mL separation funnel, which was extracted three times with 3×10 mL of ethyl acetate by shaking vigorously for 2 min each time. The organic phases were combined and evaporated to dryness in a water bath at 40 °C using a rotary evaporator (Model RE-201C, KEHUA, China) at low pressure. The residue was immediately dissolved in 10 mL of 26 mmol/L formic acid solution. The final solution was passed through a 0.22 μ m filter membrane and injected into the HPLC system (Model Agilent 1260, Palo Alto, CA, USA).

The analysis was done under the following conditions: the analytical column was Waters XBridge TM (100 $\times$ 4.6 mm i.d., 3.5 μ m C18 stationary phase); Mobile phase was acetonitrile-26 mmol/L formic acid aqueous solution (5:95, v/v) with a flow rate of 0.75 mL/min; UV detector wavelength was set at 276 nm and the injection volume was 20 μ L.

Phenolic compounds

Determination of phenolic compounds was carried out using HPLC method described by Zhang et al. (2014) with little modification. Fresh apple juices were filtered through 0.22 μ m filter membrane, and injected into the HPLC system (Model Agilent 1260, Palo Alto, CA, USA). The separation was done using a VP-ODS C18 column (250 $\times$ 4.6 mm i.d., 5 μ m particle size). The injection volume was 10 μ L. The UV detector wavelength was set at 280 nm. The mobile phase was 87 mmol/L of glacial acetic acid in water (A) and 87 mmol/L of glacial acetic acid in methanol (B). The column temperature was set at 30 °C. The gradient parameters used were as follows: elution rate of 1.0 mL/min, with (B) mL/100 mL of elution solution = 10–20 (0–5 min), 20–30 (6–20 min), 30–50

(21–30 min), 50 (31–40 min), 50–10 (41–50 min) and 10 (51–55 min).

Total phenol

Total phenol contents of apple juices were determined using a modified version of the Folin–Ciocalteu procedure described by Zhang et al. (2014). Methanol extract (200 μ L) of apple juices was mixed with 1.0 mL of Folin–Ciocalteu reagent (diluted two times) and 1.8 mL of deionized water. The reaction was terminated with 1.0 mL of sodium carbonate solution (0.1 g/mL). After 2 h of incubation at room temperature, the absorbance was read at 765 nm using a spectrophotometer (T6, Puxi, Beijing, China). The standard curve was prepared using gallic acid standard solutions of known concentration, and the results were expressed as mg gallic acid equivalent/100 mL of apple juice (mg GAE/100 mL juice).

Organic acids

The organic acids analysis carried out with 1260 HPLC system using the method reported by Rodriguez et al. (1992) with some modifications. Apple juices were passed through a 0.22 μ m filter membrane, and injected into the HPLC system. The analytical column was Zorbox C18 having the dimension of 250 $\times$ 4.6 mm i.d., 5 μ m particle size. The column temperature and UV detector wavelength were set at 25 °C and 210 nm, respectively. The injection volume was 10 μ L. The elution was done using 0.01 mol/L (NH₄)₂HPO₄ (adjusted to pH 2.6 with 3.44 mmol/L of H₃PO₄) at a flow rate of 1.0 mL/min.

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as the mean $\pm$ standard deviation (SD). Analysis of variance (ANOVA) was carried out to determine any significant difference ($P < 0.05$) among the

applied treatments using the SPSS 18.0 software (IBM, Chicago, USA).

Results and discussions

Patulin analysis

Ozone as a strong oxidant, has ability of efficiently degrading mycotoxins such as aflatoxins, patulin, zearalenone, ochratoxin, fumonsin, deoxynivalenol, etc. (McKenzie et al. 1997; Cataldo 2008; Diao et al. 2013; Savi et al. 2014). Patulin could be degraded into CO₂, oxalic acid, diglycolic acid and H₂O at high ozone concentration or enough ozonation time by attacking two conjugated ethylenic double bonds (Fig. 1) on its chemical structure (McKenzie et al. 1997; Cataldo 2008; Amado-Piña et al. 2017; de Souza Sartori et al. 2017). In this study, patulin in apple juices was significantly reduced during ozone detoxification ($P < 0.05$, Fig. 3). Patulin in the control sample (ozonation time 0 min) was 201.06 ± 2.70 µg/L, and it was decreased to 49.54 ± 2.60 µg/L after 15 min of ozonation time, and reduced by 75.36%. It was below the limit level in apple juice (50 µg/L) set by the World Health Organization (WHO 1995). At 25 min of ozonation time, patulin in apple juices reduced to 21.64 ± 0.52 µg/L, which was not detectable after 30 min of exposure time. As can be seen from Fig. 3, the degradation of patulin in apple juices followed an exponential decay curve and the correlation coefficient (R) was 0.9945, which indicated that the reduction of patulin in apple juices depended on the ozonation time. McKenzie et al. (1997) reported that 5 µg/mL of patulin in aqueous solution was completely degraded by 10% ozone within 15 s. In their study, patulin was completely degraded by ozone in a very short time (15 s) while a long time was needed to do it in our study

Fig. 3 Changes of patulin in apple juices during ozone processing within 30 min (ozone concentration 12 mg/L and flow rate 3 L/min)

(30 min). The main reasons are the different detoxification conditions, i.e. they used the higher ozone concentration (10%) to treat aqueous solution containing patulin, while 12 mg/L of ozone (equivalent to 1%) was used to treat the apple juices containing patulin in our study. Therefore, increasing ozone concentration can largely reduce the detoxification time. In addition, the nutritional components of apple juice, such as the vitamins, organic acids and phenols, may restrict the degradation of patulin by consuming partial ozone.

Total phenol and phenolic compounds

Phenolic compounds are secondary metabolites in plants with different structures and functions. They have an aromatic ring bearing one or more hydroxyl groups and varied structure from that of a simple phenolic molecule to that of a complex high-molecular mass polymer (Balasundram et al. 2006). Phenolic compounds in apple juices are mainly phenolic acids, flavonoids and their polymers based on the reported literatures (Karaman et al. 2010; Torres et al. 2011; Haminiuk et al. 2012). The chromatograms of phenolic compounds in the standard solution, fresh apple juices, and apple juices after ozone treatment for 15 min are shown in Fig. 4, and their changes during ozone detoxification are presented in Table 1. As can be seen in Fig. 4 and Table 1, 8 major phenolic compounds were identified in apple juices, which were catechin, chlorogenic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, *o*-coumaric acid, and quercetin in the order of their retention times. The contents of phenolic compounds in fresh apple juices used in this study were in the ranges of reported literatures (Karaman et al. 2010; Torres et al. 2011). It is obvious that ozone detoxification seriously destroyed the phenolic compounds in fresh apple juices with the increase of ozonation time (Table 1). When ozonation time was at 10 min, all the phenolic compounds in apple juices were reduced by more than half of their initial concentrations, and the losses of quercetin, ferulic acid and *p*-coumaric acid reached 71.38%, 79.10%, and 78.55%, respectively. At ozonation time of 15 min, the ferulic acid and *p*-coumaric acid in apple juices could not be detected, and then *o*-coumaric acid and caffeic acid disappeared at 20 min. Seven phenolic compounds in apple juices were completely destroyed by ozone and only a small amount of chlorogenic acid remained (7.92 ± 0.58 mg/L) at 25 min of ozonation time.

The total phenol content of fresh apple juices was 692.36 ± 49.81 mg GAE/100 mL in this study, which was also in the range of reported literatures (Lobo et al. 2009; Patil et al. 2010). It is also found to be significantly decreased with the increase of ozonation time ($P < 0.05$, Table 1), and it was decreased to 297.12 ± 15.92 mg

Fig. 4 HPLC chromatograms of phenolic compounds in standard solution (a), fresh apple juices (b), and apple juices after ozone processing for 15 min (c)

Table 1 Changes of phenolic compounds and total phenol in apple juices during ozone processing

Phenolic compounds	Concentration of phenolic compounds at different ozone processing times (mg/L)					
	0 min	5 min	10 min	15 min	20 min	25 min
Chlorogenic acid	278.43 ± 21.72 ^a	203.15 ± 16.97 ^b	109.43 ± 9.85 ^c	57.19 ± 3.57 ^d	18.24 ± 1.29 ^e	7.92 ± 0.58 ^f
Quercetin	47.59 ± 2.43 ^a	30.38 ± 2.47 ^b	13.62 ± 0.49 ^c	4.12 ± 0.46 ^d	0.37 ± 0.08 ^e	ND
Epicatechin	42.85 ± 2.25 ^a	34.94 ± 2.30 ^b	21.27 ± 1.72 ^c	8.44 ± 1.01 ^d	1.23 ± 0.26 ^e	ND
<i>o</i> -Coumaric acid	35.33 ± 0.96 ^a	24.57 ± 1.12 ^b	11.64 ± 0.31 ^c	1.73 ± 0.33 ^d	ND	ND
Caffeic acid	28.10 ± 1.59 ^a	19.63 ± 0.96 ^b	11.98 ± 1.42 ^c	2.45 ± 0.29 ^d	ND	ND
Catechin	23.91 ± 2.64 ^a	17.25 ± 1.02 ^b	10.51 ± 0.97 ^c	6.38 ± 0.72 ^d	0.98 ± 0.07 ^e	ND
Ferulic acid	16.22 ± 1.27 ^a	11.83 ± 0.65 ^b	3.39 ± 0.53 ^c	ND	ND	ND
<i>p</i> -Coumaric acid	8.16 ± 0.73 ^a	5.36 ± 0.5 ^b	1.75 ± 0.38 ^c	ND	ND	ND
Total phenol (GAE, mg/100 mL)	692.36 ± 49.81 ^a	515.35 ± 37.23 ^b	361.93 ± 22.45 ^c	297.12 ± 15.92 ^d	208.75 ± 12.47 ^e	136.41 ± 9.64 ^f

“ND” stands for “Not detectable”

Different letters indicate the significant difference at $P < 0.05$ level between each ozone processing time in the same row

Fig. 5 HPLC chromatograms of organic acids in standard solution (a), fresh apple juices (b), and apple juices after ozone processing for 15 min (c)

GAE/100 mL at the ozone concentration of 12 mg/L for 15 min with a reduction of 57.09%. So the reduction of total phenol has further verified the losses of phenolic compounds in apple juices.

Patil et al. (2010) and Torres et al. (2011) had reported the similar results that ozone processing significantly influenced the phenolic compounds and total phenol content in apple juices. The chlorogenic acid, caffeic acid, cinnamic acid, and total phenol content of apple juices were reduced by 224.15 mg/L, 17.88 mg/L, 129.75 mg/L and 317 mg

GAE/100 mL when exposed to 4.8% (w/w) ozone for 10 min, respectively. Also, their reductions were all dependent on the ozonation time.

The losses of phenols are due to the direct or indirect oxidative roles of ozone. The former occurs by the ozone molecule based on the Criegee mechanism and the latter by the production of hydroxyl radicals (Cullen et al. 2009; Diao et al. 2012; Amado-Piña et al. 2017).

Table 2 Changes of organic acids in apple juices during ozone processing

Organic acids	Concentration of organic acids at different ozone processing times (mg/L)						
	0 min	5 min	10 min	15 min	20 min	25 min	30 min
Shikimic acid	72.81 ± 1.46 ^a	71.94 ± 0.14 ^a	69.82 ± 0.61 ^b	67.79 ± 0.56 ^c	63.16 ± 0.45 ^d	62.38 ± 0.46 ^d	62.03 ± 0.72 ^d
Acetic acid	32.65 ± 3.79 ^a	32.96 ± 2.70 ^a	33.69 ± 0.95 ^{ab}	34.32 ± 1.60 ^b	44.62 ± 2.41 ^c	52.01 ± 3.52 ^d	74.28 ± 2.19 ^e
Succinic acid	222.27 ± 20.08 ^{ab}	240.82 ± 6.42 ^a	205.79 ± 6.37 ^b	191.05 ± 8.62 ^b	176.65 ± 5.08 ^b	166.06 ± 1.82 ^{bc}	160.84 ± 2.82 ^c
Maleic acid	2.98 ± 0.05 ^a	3.03 ± 0.03 ^a	2.98 ± 0.01 ^a	3.01 ± 0.05 ^a	3.34 ± 0.05 ^b	3.39 ± 0.01 ^b	3.56 ± 0.01 ^c
Malic acid	162.62 ± 6.06 ^a	93.66 ± 1.06 ^b	53.67 ± 7.08 ^c	ND	ND	ND	ND
Total	493.33 ± 6.29 ^a	442.41 ± 2.07 ^b	365.95 ± 3.00 ^c	296.17 ± 2.71 ^d	287.77 ± 2.00 ^{de}	283.84 ± 1.45 ^e	300.71 ± 1.44 ^d

“ND” stands for “Not detectable”

Different letters indicate the significant difference at $P < 0.05$ level between each ozone processing time in the same row

Organic acids

Presently, the changes of organic acids in fruit juices during ozone processing have not been reported except for ascorbic acid. Cullen et al. (2009) summarized that ozone processing could significantly reduce the ascorbic acid in different juices. The organic acids in apple juices are very important for their flavor, nutrition, acceptability, and shelf life. Therefore, to reduce the losses of organic acids in apple juice processing should be considered by processors. In the reported literatures, the main organic acids in apple juices include malic acid, ascorbic acid, fumaric acid, succinic acid, shikimic acid, acetic acid and maleic acid (Shui and Leong 2002; Chinnici et al. 2005). A total of five organic acids was detected in apple juices, which were shikimic acid, acetic acid, succinic acid, maleic acid, and malic acid in the order of their retention times (Fig. 5). Their changes during ozone detoxification are shown in Table 2. As can be seen in Table 2, succinic and malic acids were the major organic acids with a ratio exceeding 78% of total acids in apple juices. With the increase of ozonation time, the contents of malic, succinic and shikimic acids were decreased while those of acetic and maleic acids were increased. Within 5 min of ozonation time, four of the five organic acids had no significant changes except for the malic acid ($P > 0.05$). At 15 min of ozonation time, malic acid could not be detected, and succinic and shikimic acids were significantly decreased, which reduced by 14.05% and 6.89%, respectively. After 15 min of ozonation time, the contents of succinic and shikimic acids were slowly decreased while that of acetic acid was obviously increased ($P < 0.05$). During ozone detoxification, the acetic acid concentration of apple juices still increased, which corresponded to the reduction in malic, succinic and shikimic acids, indicating a large amount of acetic acid was produced from the ozonolysis of

the three acids. The increase of maleic acid in apple juices was very slow, and the increased maleic acid might be an oxidation product from the degradation of shikimic acid by ozone (Bailey 1958). Additionally, the structure of malic acid is very similar to that of succinic acid, while the degradation rate of the former was faster than that of the latter. The reason is that the malic acid molecule contains a hydroxyl group on the α -carbon atom, which is easily attacked by ozone (Glaze 1986).

Based on the results, phenolic compounds and organic acids present in apple juices can reduce the detoxification efficiency of OP by consuming partial ozone, which verifies the hypothesis mentioned above that the nutritional components of apple juices may restrict the ozonolysis of patulin. While they are good at the flavor and taste of apple juices, the OP must be optimized to overcome its adverse effects on quality of apple juices. There are three suggestions: (1) OP is used in the detoxification of apple juices containing less patulin. The range of patulin concentration in apple juices for using OP will be studied in the next work. (2) A moderate OP may be a good choice to reduce its effects on the quality of apple juices. For example, higher ozone concentration and shorter ozonation time or lower ozone concentration and longer ozonation time with interval detoxification. (3) When OP is used to detoxify apple juices with higher patulin concentration, the losses of phenolic compounds and organic acids can be supplemented by adding the corresponding nutritional and functional components.

Conclusion

OP was observed to effectively degrade patulin in apple juices, while resulting in significant losses in the phenolic compounds and organic acids responsible for the flavor and

taste of apple juices. The adverse effect of OP on the quality of apple juices maybe considered while adopting this detoxification technique. The toxicological and sensory evaluations to define the safety and quality differences of apple juices treated by OP need to be explored. The use of OP as an alternative to clarification technique for detoxifying patulin in apple juices may be considered as a better choice based on its higher detoxification efficiency, lower costs, and without environmental pollution in practical application.

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