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# High hydrostatic pressure assisted degradation of patulin in fruit and vegetable juice blends

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ABSTRACT

The degradation of patulin introduced into different juices then treated with high hydrostatic pressure (HHP) was evaluated. A model juice prepared from apple and spinach (AS) was studied along with commercially available apple-based beverages; Pineapple:Apple:Mint (PAM), Apple:Carrot:Beet:Lemon:Ginger (CAB) and Romaine:Celery:Cucumber:Apple:Spinach:Kale, Parsley:lemon (GJ). The extent of patulin degradation was found to be dependent on applied pressure and processing time (degradation rates ranged from 0.04 to 0.19 ppb/s). The extent of patulin degradation could also be significantly ($P < 0.05$) correlated with the sulfhydryl group concentration of the juice with ascorbic acid and nitrite being less significant. HHP treatment of 600 MPa for 300 s at 11 °C resulted in a 62 ppb decrease in patulin introduced into GJ juice which also contained the highest level of thiols (97 μM). The thiol concentration of the other juices ranged between 39 and 69 μM with a corresponding decrease in patulin of 43–49 ppb following the same HHP treatment. The study has illustrated that HHP can be applied as a risk management tool to control patulin in apple based beverages although the extent of mycotoxin degradation is dependent on processing conditions and composition of the juice.



## 1. Introduction

Patulin is a mycotoxin of concern in beverages due to its widespread distribution and toxicity (Moake, Padilla-Zakour, & Worobo, 2005). The mode-of-toxicity is thought to be through disruption of cellular function via interaction of the C-6 and C-2 of the unsaturated heterocyclic lactone of patulin with sulfhydryl groups of proteins, enzymes and other cellular components (Pfeiffer, Diwald, & Metzler, 2005; Schumacher, Mueller, Metzler, & Lehmann, 2006). Apple based products are commonly linked to patulin although the producing mould can be encountered in a range of vegetable and fruit types (Yang, Tang, Li, Zhang, & Hu, 2014). The level of concern for patulin in apple based beverages is 50 ppb with a tolerable daily intake of 43 μg/kg body weight (Moake et al., 2005). The prevalence of patulin is dependent on the presence of producing moulds (e.g. *Penicillium expansum*) and climatic conditions with warm, humid, weather promoting mould growth (Morales, Marin, Ramos, & Sanchis, 2010). Patulin associated with apple products in excess of the 50 ppb limit is estimated to be >10% in North America and is predicted to increase due to climate change that would be conducive to mould growth (Harris, Bobe, & Bourquin, 2009; Paterson & Lima, 2010; Yang, Li, et al., 2014; Yang et al., 2014). Given prevalence of patulin there is a need for interventions to decrease levels of the mycotoxin in juices to below the 50 ppb regulatory limit. Methods to date have included ammonia based treatments, ozone, biological degradation and physical methods such as UV (Cataldo, 2008; Celli, Coelho, Wosiacki, & Garcia-Cruz, 2009; Coelho et al., 2008). From the aforementioned methods, UV is the most practical although is limited to clear juices with high UV-C transmission (Zhu, Koutchma, Warriner, & Zhou, 2014). However, juices with low UV transmission are incompatible with ultraviolet based methods and hence alternative pasteurization techniques are required. Currently, the majority of non-thermally processed cold-pressed juices are treated using high hydrostatic pressure (HHP) with the primary aim of pathogen reduction and shelf-life extension (Buzrul, Alpas, Largeteau, & Demazeau, 2008; Erkmen & Dogan, 2004). However, it has also been reported that HHP treatment can also degrade patulin levels in apple juice and concentrate. Bruna, Voldrich, Marek, and Kamarad (1999) reported that HHP treatments of 300 MPa, 500 MPa, and 800 MPa at 20 °C for 60 min led to a reduction of original patulin content (155 mg/kg) by 16, 20, and 23%

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in apple concentrate, and 42, 53, and 62% in apple juice, respectively. Avsaroglu, Bozoglu, Alpas, Largeteau, and Demazeau (2015) reported up to a 24% decrease in patulin spiked into clear apple juice originally containing 100 ppb before processing for 5 min a 300 MPa. The authors noted that the efficacy of HHP treatment in degrading patulin was highly variable although the general trend was an increase in degradation rate with pressure in the range of 300–500 MPa (Avsaroglu et al., 2015). The underlying reason for the high variation was unknown but it is possible that the composition of the juice was a contributory factor. It is well established that the lactone ring of patulin is susceptible to nucleophilic attack by oxidizing agents such as ascorbic acid thereby neutralizing toxicity over time (Alves, Oliveira, Laires, Rodrigues, & Rueff, 2000). Patulin can also react with thiols that results in breakage of the lactone ring resulting in adducts with reduced toxicity (Appell, Jackson, & Dombrink-Kurtzman, 2011). It follows that patulin could further react with different constituents within fruit and vegetable juices, for example nitrites, that could be enhanced under high pressure. Therefore, the aim of the current study was to correlate the extent of high pressure assisted patulin degradation with HHP treatment parameters and juice composition.

## 2. Materials and methods

### 2.1. Reagents

Patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one), acetonitrile (ACN), methanol, ethyl acetate, acetic acid, metaphosphoric acid, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), ascorbic acid and sodium nitrite were purchased from Sigma–Aldrich (St. Louis., MO, USA). Griess Reagent for Nitrite determination was purchased from Abcam Inc (Cambridge, MA, USA).

### 2.2. Model fruit and vegetable beverage preparation

Apple:spinach (AS) juice was used as a model system with defined composition. AS was prepared from salad spinach (200 g) obtained from a local supermarket and blended with 1 L of water. The homogenate was then centrifuged at 5000 × g for 10 min to remove large particulates prior to adding apple juice concentrate (71 °Brix) to give a final brix of *ca*. 6. The pH of the juice was then adjusted to 3.5 using lemon juice. Commercially produced vegetable and fruit blends were provided by Blue Print Cleanse (NY, USA). PAM (Pineapple, Apple and Mint), CAB (Apple, Carrot, Beet, Lemon and Ginger), and GJ (Romaine, Celery, Cucumber, Apple, Spinach, Kale, Parsley and lemon).

### 2.3. Juice characterization

The pH values of the different juices were measured using a pH meter (model 868, Thermo Orion, MA, USA). The ascorbic acid content was determined according to the method described by Patil (Patil, Vanamala, & Hallman, 2004). Briefly, the sample was homogenized with extraction solution (3% v/v metaphosphoric acid and 2% v/v acetic acid). The resulting mixture was centrifuged and the supernatant filtered through a 0.45 μm membrane filter and analyzed by HPLC at 254 nm (Agilent Technologies 1200 Series, Palo Alto, CA). A Phenomenex Phenosphere C18 column with 3 μm particle size (150 × 4.6 mm) with a C18 guard column (Torrance, CA, USA). The chromatographic separation was performed with an isocratic mobile phase of 0.1% v/v metaphosphoric acid at 1 ml/min. The sulfhydryl content in samples was determined spectrophotometrically at 420 nm using a UV–Vis plate reader (EL 340, Bio-Tek Instruments Inc., Winooski, VT, USA). Sulfhydryl content was measured by monitoring the increase in absorbance due to the formation of 5-thio-2-nitrobenzoic acid (NTB) from 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (Riener, Kada, & Gruber, 2002). The reaction mix consisted of 150 μl reagent solution (8 ml of 100 mM potassium phosphate buffer, pH 7.0 and 228 μl 1.5 mg/ml DTNB in DMSO) into which 10 μl sample was added before incubating at room temperature (24 °C) for 5 min and finally determining the absorbance at 420 nm. BSA was used to construct a standard curve.

Nitrite concentration was determined by Griess Reagent for Nitrite Assay Kit Colorimetric by Abcam Inc. (Cambridge, MA, USA) at 540 nm. For nitrite determination, 80 μl sample solution and 20 μl buffer solution was mixed in a 96-well plate along with 50 μl of Griess Reagent A. After 5 min, 50 μl of Griess Reagent B was added to each well, and mixed. The mixture was incubated for 10 min at room temperature before reading the absorbance at 540 nm.

### 2.4. High hydrostatic pressure processing treatment

The juices were dispensed in 25 ml aliquots into pouches and subsequently spiked with the appropriate concentration of patulin. Pressure treatment was performed in a 51 L capacity Hyperbaric unit (Hyperbaric, Burgos, Spain) and ranged from 400 to 600 MPa with processing times of 0–300 s. The temperature of the incoming water was 11 °C.

### 2.5. Extraction and quantification of patulin

Aliquots of 150 μl pectinase enzyme solutions (3800 units/ml) were added to 20 ml juice sample that were subsequently incubated for 2 h at 40 °C and then centrifuged at 4500 × g for 5 min. The patulin was extracted from the supernatant of the centrifuged juice using SPE method (Eisele & Gibson, 2003). The SPE cartridge (Oasis HLB extraction cartridge, Waters Corp., Milford, MA) was conditioned by passing 2 × 3 ml of methanol through the column and then equilibrated with 2 × 3 ml of water before loading 2.5 ml of the juice sample. The cartridge was then washed using 2 × 3 ml of 1% v/v acetic acid solution. Patulin was eluted with 1 ml ethyl acetate and then evaporated to dryness under a stream of nitrogen at 40 °C. The dried extract was resuspended in 1 ml of 0.1% v/v acetic acid solution.

Patulin was quantified using RP-HPLC (Agilent Technologies 1200 Series, Palo Alto, CA) equipped with a quaternary pump, an online degasser and a wavelength set at 276 nm. A Phenomenex Phenosphere C18 column with 3 μm particle size (150 × 4.6 mm) with a C18 guard column (Torrance, CA, USA) was used as the separation column. The mobile phase consisted of 5% v/v acetonitrile and 0.05% v/v acetic acid in distilled water at a flow rate of 1 ml/min with run time for 20 min.

### 2.6. Data analysis

All the experiments were performed with three replicates. The results were expressed as mean values and standard deviation (SD). The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test with $\alpha = 0.05$. The analysis was carried out using SPSS Statistics 17.0 (IBM Corporation, Armonk, New York, USA.). The screening of models and the estimation for model parameters were conducted using Matlab R2011a (Math Works Inc., Natick, Massachusetts, USA). The surface response analysis was carried out using Design Expert 7.0 (Stat-Ease, Inc., Minneapolis, Minnesota, USA).