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# Natural occurrence of patulin in different fruits, juices and smoothies and evaluation of dietary intake in Punjab, Pakistan

Shahzad Zafar Iqbal [a, *], Shabana Malik [a], Muhammad Rafique Asi [b], Jinap Selamat [c, d], Noeen Malik [e]

[a] Department of Applied Chemistry, Government College University Faisalabad, 38000, Pakistan
[b] Food Toxicology Lab., Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Faisalabad 38950, Pakistan
[c] Faculty of Food Science & Technology, Universiti Putra Malaysia, Serdang, 43400, Malaysia
[d] Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, 43400, Malaysia
[e] Department of Radiology, School of Medicine, New York University, USA

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ABSTRACT

The current study is focused to investigate the natural presence of Patulin (PAT) in various fruits, juices and smoothies marketed and consumed in Pakistan. A total of 237 samples of fruits, juices and smoothies were analysed using isocratic HPLC with UV detector. The results have shown that 136 out of 237 (57.4%) samples have found positive with PAT contamination with levels ranged from 0.04 to 1100 μg $kg^{-1}$. The highest mean level of PAT was found in red globe grapes i.e. 921.1 ± 22.4 μg $kg^{-1}$. About 33.8% samples have levels of PAT with concentration above the permissible regulations of European Union (EU) i.e. 50 μg $kg^{-1}$. The dietary intake of PAT from fruits, juices and smoothies were 0.0049, 0.0016 and 0.0014 μg $kg^{-1}$ bw $day^{-1}$ with hazard quotient (HQ) value of 1.22, 0.40 and 0.35%, respectively. The incidence and occurrence of PAT in fruits and fruits products have high and need urgent attention for comprehensive survey. The results of present study would be useful for farmers, traders, and consumers.


## 1. Introduction

Mycotoxins are recognized as secondary metabolites, which mainly produced by specific filamentous fungi under appropriate conditions of temperature and humidity (Nielsen, Mogensen, Johansen, Larsen, & Frisvad, 2009; Iqbal, Paterson, Bhatti, & Asi, 2011). PAT (a polyketide lactone 4-hydroxy-4H-furo (3,2c) pyran-2 (6H)-one, PAT) is toxic class of mycotoxins (Ji et al., 2017) and it is a toxic metabolite of at least 60 different species of fungi such as *Penicillium expansum* (*P. leucopus*), *P. crustosum*, *P. patulum* (*P. urticae, P. griseofulvum*), and *A. clavatus* (Wright, 2015). The fungus *Penicillium expansum* is the most commonly PAT producing (Moake, Padilla-Zakour, & Worobo, 2005). The PAT producing fungi have been isolated from various fruits and vegetables including apples, plums, peaches, pears etc. (Piqué, Vargas-Murga, Gómez-Catalán, Lapuente, & Llobet, 2013). However, due to its toxicity it was classified as mycotoxin in 1960s (Puel, Galtier, & Oswald, 2010). Previous studies have shown that PAT could induce acute symptoms like convulsions, agitation, ulceration, edema, intestinal inflammation and vomiting, and could cause chronic neurotoxic, immunotoxic, genotoxic and teratogenic effects in rodents (Boon, Baars, Van Klaveren & Van Rossum, 2009; Spadaro, Ciavorella, Frati, Garibaldi, & Gullino, 2007).

International Agency for Research on Cancer (IARC) has classified PAT in Group 3, not carcinogen to human (IARC, 2002). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) implemented a provisional maximum tolerable daily intake (PMTDI) of 0.4 μg $kg^{-1}$ body weight (bw) $day^{-1}$ for PAT (WHO, 1995, p. 36). Several countries and organizations have set maximum limits for PAT in food to control health risk hazards. The Codex Alimentarius Commission (2003) has established a maximum level of 50 μg $kg^{-1}$ for PAT in apple juice and the European Union (EU) adopted a maximum level for PAT at 50 μg $kg^{-1}$ in fruit juices, concentrated fruit juices, fruit nectars, as well as spirit drinks, cider and other fermented drinks derived from apples or containing apple juice; 25 μg $kg^{-1}$ in solid apple products; and 10 μg $kg^{-1}$ in apple-based products and baby foods, other than processed cereal

* Corresponding author.
*E-mail addresses:* shahzad10542005@yahoo.com, shahzad@gcuf.edu.pk (S.Z. Iqbal).

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based foods, for infants and young children (Commission Regulation, 2006). However, no regulations exist for PAT in Pakistan.

Considering the above mentioned facts, the current study has focused to investigate the incidence of PAT in different fruits, juices and smoothies, to compare the incidence levels of PAT in fruits and juices with the EU maximum limit and to investigate the dietary intake of PTA in local population. There is no single report of PAT in fruit juices from Pakistan and the results of present study will be useful to access the current status of this toxin in fruits and juices.

## 2. Materials and methods

### 2.1. Sampling

Total 237 samples of fruits, juices and 42 samples of smoothies have been collected from major cities of Punjab (Lahore, Faisalabad, Multan, Islamabad), Pakistan during January 2017 till March 2017. The samples were collected randomly and the samples size of fruits was 1 kg, while the size of juice sample was not less than 1 L each. Samples were kept in plastic bags and stored in a refrigerator at −4 °C until analysis in their original packages.

### 2.2. Regents and chemicals

The standard of PAT (100 μg $mL^{-1}$) in acetonitrile was purchased from Sigma Aldrich (Saint-Louis, Mo., USA). All reagents like acetic acid, ethyl acetate and sodium carbonate were obtained from Merck and Sigma-Aldrich (France). HPLC grade solvents such as acetonitrile and methanol were purchased from Fisher Scientific (Fisher chemicals, France). A standard curve for PAT was prepared by diluting the standard with methanol into concentrations of 10, 50, 100, 150, 200, 250 and 300 μg $L^{-1}$ solutions and stored in caped vials in a refrigerator at −4 °C, until further analysis. All other reagents were at least of analytical grade.

### 2.3. Extraction and cleanup of samples

Extraction of PAT from fruits and juices were performed according to the method described by Zouaoui, Sbaii, Bacha & Abid-Essef, (2015) using HPLC with UV detector with some modifications. Sample of 50 mL (liquid) or 50 g of solid was added to 100 ml of water and extracted with 50 mL of ethyl acetate by mixing vigorously for 15 min using a vortex mixer. After vortex, the mixture was centrifuged for 10 min at 4500 rpm. The organic upper layer was transferred to a centrifuge tube and the aqueous phase was twice re extracted with 20 mL of ethyl acetate. The organic layers were combined and 2 mL of 1.5% sodium carbonate was added and the tube was mixed vigorously. Then 5 mL of ethyl acetate was added to $Na_2CO_3$ solution and shacked vigorously for 5 min. The pH of solution was adjusted to 4 using glacial acetic acid. The solution was evaporated to dryness at 60 °C and 5 mL of acetonitrile solution (5%) was added to the residue and the reconstituted extract was purified through a 0.22 μm syringe filter (Millipore) and again evaporated to dryness at 60 °C. Finally, 500 μL of methanol was added to residue and 20 μL injected for HPLC analysis.

### 2.4. HPLC conditions

The analysis of PAT in fruit and juices were performed on HPLC (Shimadzu LC-10A series, Kyoto, Japan), equipped with UV detector at 276 nm. The column was a C18 (4.6 × 250 mm, 5 μm) Discovery of Supelco, Bellefonte, USA. The mobile phase acetonitrile and water (90:10 v/v) was used with a flow rate of 1 mL $min^{-1}$, isocratically.

### 2.5. Estimation of dietary intake

The estimated dietary intake of PAT in fruits and juices were calculated by following the method of Iqbal, Asi, and Malik (2017). The dietary intake level of PAT in consumers was estimated based on a food frequency questionnaire involving 400 male individuals. The recalled period of fruits and juices consumption was of three weeks. To estimate the amount, the information about bowl, glass and cups were used to activate the interviewees' memory and the information was collected regarding the weight of the consumed fruits/juices.

$$\text{Dietary intake ng/L/day} = \frac{\text{Daily intake of } \frac{\text{fruits}}{\text{juices}} \left(\frac{g}{day}\right) x \text{ PAT in } \frac{\text{fruits}}{\text{juices}} \left(\frac{ng}{L}\right)}{\text{Average individual weight } (kg)}$$

### 2.6. Statistical analysis

The data was presented as mean ± standard deviation and three replicate of each sample was analysed. The simple linear correlation and regression analyses were used to find out the coefficient of determination ($R^2$) using SPSS (IBM PASW statistics 19 software, USA).

## 3. Results and discussion

### 3.1. Method validation and quality control parameters

The method validated was evaluated in terms of recovery analysis, linearity, reproducibility, repeatability, and determination of the limits of detection (LD) and limit of quantification (LQ) of PAT in various fruit products consumed and marketed in Punjab, Pakistan. To examine the recovery rate, blank samples were spiked with three known concentration of PAT (50, 100 and 200 μg $mL^{-1}$). The mean recovery values were varied from 85.5 to 93.7%, with the average relative standard deviation (RSD) varied from 7 to 18%. Linearity was confirmed using a 7 point calibration curve for each PAT concentration with concentration varied from 10 to 300 μg $L^{-1}$. The curve was linear with coefficient of determination ($R^2$) value of 0.9996. The limit of detection (LD) was determined as 3:1 of signal-to-noise ratio obtained with free sample and LQ was calculated as 10:1 signal-to-noise ratio. The LD and LQ of PAT were 0.04 and 0.12 μg $kg^{-1}$, respectively. Fig. 1 represents the standard chromatogram of PAT (Fig. 1a), and natural occurrence of PAT in apple sample (Fig. 1b).

### 3.2. Incidence of PAT

The incidence and occurrence of PAT in fruits, juices and smoothies are shown in Table 1. The results have shown that 136 (57.4%) out of 237 samples of fruits, juices and smoothies were found positive with PAT. The highest mean level of PAT was found in red globe grapes 921.1 ± 22.4 μg $kg^{-1}$, with levels ranged from 0.04 to 1100 μg $kg^{-1}$. The fruits juices (branded) have shown lowest levels of PAT. Furthermore, the smoothie's samples were also observed less contaminated with PAT, with concentration ranged from 0.04 to 250. 6 μg $kg^{-1}$.

The incidence and levels of PAT in fruits, juices and smoothies greater than the EU recommended limit are shown in Table 2. The results have shown that 33.8% samples were found levels of PAT with concentration higher than the recommended limit of EU. However, 42.2% samples were found levels of PAT below the LOD.

The results of current study were comparable with the findings