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Short communication

## Incidence of patulin in apple juice concentrates produced in Turkey

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### Abstract

A total of 215 apple juice concentrate samples from three different producers in Turkey were analyzed for patulin using reversed-phase high-performance liquid chromatography. The detection limit of patulin in single strength apple juice at a sugar content of 11.2°Brix was lower than 5 µg/l. Patulin was detected in all of the samples analyzed, at concentrations ranging from 7 to 376 µg/l. Of the samples, 43.5% were found to exceed a patulin contamination level of 50 µg/l. © 1998 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Patulin belongs to a class of toxic compounds known as mycotoxins. All mycotoxins are secondary metabolites of filamentous fungi and are undesirable in foods. Not all filamentous fungi are capable of producing mycotoxins.

Patulin {4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one} is produced by several species of *Penicillium* [*Penicillium expansum* (*P. leucopus*), *P. patulum* (*P. urticae*, *P. griseofulvum*), *P. calavrforme*, *P. melinii*, *P. equinum* (*P. terrestre*), *P. roqueforti*, etc.], *Aspergillus* (*Aspergillus clavatus*, *A. giganteus*, *A. terreus*) and *Byssochlamys* (*Byssochlamys nivea* and *B. fulva*) [1,2].

Of the fungi capable of producing patulin, *Penicillium expansum* is probably the most commonly encountered specimen. This specimen is often isolated from decaying apples. Patulin has mainly been found in apples and apple products and, occasionally,

it has been determined in silage, cereals, bread, cheese and in pears, apricots, peaches and grapes, as well as in products derived from these fruits [3].

Patulin can be used as an indicator of the quality of processed fruit juices and fruit products, since an appreciable concentration of the mycotoxin remains in the product after processing.

Concern centers around patulin's properties as a compound that can produce both acute toxicity problems as well as its potential as a carcinogen. Lack of mutagenic activity by patulin in short-term mutagenic assay systems has also been noted [4,5].

Several countries regulate patulin at levels ranging from 20 to 50 µg/l in fruit juices and other fruit products. The World Health Organization (WHO) has also established 50 µg/l as the recommended limit in apple juice [6–8].

The objective of this study was to screen apple juice concentrates produced in Turkey for patulin content. In this way, the applicability of the method published earlier by us [8] was also tested, by analyzing large number apple juice concentrates from different producers.

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## 2. Experimental

### 2.1. Materials

A total of 215 samples of apple juice concentrate (°Brix: min. 69.6 and max. 76.2), collected from three different companies in Turkey through the entire processing season between September 1996 and January 1997 were used in this study. Samples were stored below 4°C prior to analysis, diluted to 11.2°Brix upon opening and were analyzed immediately.

### 2.2. Apparatus

(a) High-performance liquid chromatograph. A Varian Model 9010 liquid chromatograph was used. It was equipped with a Rheodyne Model 7161 six-way injector with a 10- $\mu$ l loop and a Varian Model 9050 variable-wavelength UV–Vis detector set at 276 nm. The chromatograms were recorded by using a Varian Model 4400 integrator with a chart speed of 1.0 cm/min.

(b) Column. The analytical column (150 $\times$ 4 mm I.D.) was made of stainless steel and was packed with 5  $\mu$ m Elsi Sphere C<sub>18</sub> stationary phase and operated at ambient temperature. It was protected by a microparticulate MikroPak C<sub>18</sub> guard column (40 $\times$ 4 mm I.D.).

(c) Mobile phase. Water–acetonitrile (99:1, v/v) was used at a flow-rate of 1.0 ml/min. It was filtered through a 0.45- $\mu$ m regenerated cellulose acetate membrane and degassed ultrasonically just before analysis by high-performance liquid chromatography (HPLC).

### 2.3. Analytical procedure

Apple concentrate was diluted to 11.2°Brix, corresponding to single strength apple juice. Patulin determination was carried out as in ref. [8]. A 5-ml volume of single-strength apple juice was extracted twice with 10 ml of ethyl acetate by shaking vigorously for 1 min using a vortex mixer. Organic phases were combined and extracted with 2 ml of 1.5% sodium carbonate solution by shaking for 1 min. Then, the phases were allowed to separate and the aqueous phase was immediately extracted with 5

ml of ethyl acetate by shaking for 1 min. Combined organic phases were dried over 2.5 g of anhydrous sodium sulfate. Subsequently, the dried extract was filtered through a black band filter paper to remove the remaining particles of anhydrous sodium sulfate. A 2-ml excess of ethyl acetate was added to wash the filter cake layer and the filtrate obtained was combined with the filtered extract. Then the extract was evaporated just to dryness in a water bath at 40°C under a gentle stream of nitrogen. The residue was immediately dissolved in 500  $\mu$ l of water that had been acidified with acetic acid (pH 4.0) and 10  $\mu$ l of this solution were injected into the column. Final solutions were kept in a deep freezer until the chromatographic measurements.

A stock standard solution of patulin was prepared by dissolving 5 mg of pure crystalline patulin (Merck) in 25 ml of ethyl acetate. A 100- $\mu$ l volume of this solution was transferred to a 10-ml volumetric flask and evaporated just to dryness under a stream of nitrogen at room temperature. The residue was immediately dissolved in 10 ml of water that had been acidified (pH 4.0) with acetic acid. Working standard solutions were prepared by appropriate dilution of this solution with water that had been acidified with acetic acid (pH 4.0).

Recovery of patulin in apple juice ranged from 94.2 to 125.5% for spiking levels of 25, 50, 100 and 200  $\mu$ g/l, with two replicates for each spiking level. The sensitivity achieved was sufficient to allow detection at levels as low as 4  $\mu$ g/l patulin in single-strength apple juice. The residue data were not corrected for recovery.

### 2.4. Calculation of results

The amount of patulin in the final solution was determined by using a calibration graph of concentration vs. peak height and expressed as  $\mu$ g/ml. The patulin content ( $C$ ) of the apple juice was found by using the equation

$$C(\mu\text{g/l}) = \frac{1000C_{\text{spl}}V}{m}$$

where  $C_{\text{spl}}$  is the concentration of patulin in the final solution ( $\mu$ g/ml),  $V$  is the total volume of the final

solution (ml), and  $m$  is the volume of apple juice taken for extraction (ml).

### 3. Results and discussion

A total of 215 samples of apple juice concentrate, diluted to 11.2°Brix as single-strength apple juice, were analyzed for patulin content. The results of the patulin analyses of 215 diluted apple juice concentrates are presented in Table 1.

Retention time of patulin was ca. 13 min with a R.S.D. of 0.90%, indicating reproducible results. Patulin could be resolved from 5-hydroxymethylfurfural (5-HMF), which is the most important interfering co-extractive of apple juice. These results were in very good agreement with our previous results [8], and expanded the applicability data of the method. Fig. 1 illustrates the HPLC chromatograms of patulin in apple juice.

Patulin was detected in 215 samples at concentrations ranging from 7 to 376  $\mu\text{g}/\text{l}$ . Patulin levels exceeded 50  $\mu\text{g}/\text{l}$  in 46% of all samples analyzed. Different results were obtained in our previous investigation of patulin contamination in commercial apple juices marketed in Ankara through 1993–1994 [7].

In that study, the concentration of patulin was found to exceed 50  $\mu\text{g}/\text{l}$  in 11% of the apple juice samples (out of a total of twenty samples that were analyzed). These two studies indicate both a high incidence and range of patulin levels in apple juice concentrates and commercial apple juices in Turkey.

Table 1

Patulin contamination in apple juice concentrates processed by different producers<sup>a</sup>

Producer	Number (%) of samples containing patulin	
	$\leq 50 \mu\text{g}/\text{l}$ patulin	$> 50 \mu\text{g}/\text{l}$ patulin
A <sup>b</sup>	24 (36%)	43 (64%)
B <sup>c</sup>	51 (64%)	29 (36%)
C <sup>d</sup>	42 (62%)	26 (38%)

<sup>a</sup>The results are calculated based on single strength apple juice at 11.2°Brix.

<sup>b</sup>The maximum concentration was 376  $\mu\text{g}/\text{l}$ .

<sup>c</sup>The maximum concentration was 341  $\mu\text{g}/\text{l}$ .

<sup>d</sup>The maximum concentration was 130  $\mu\text{g}/\text{l}$ .

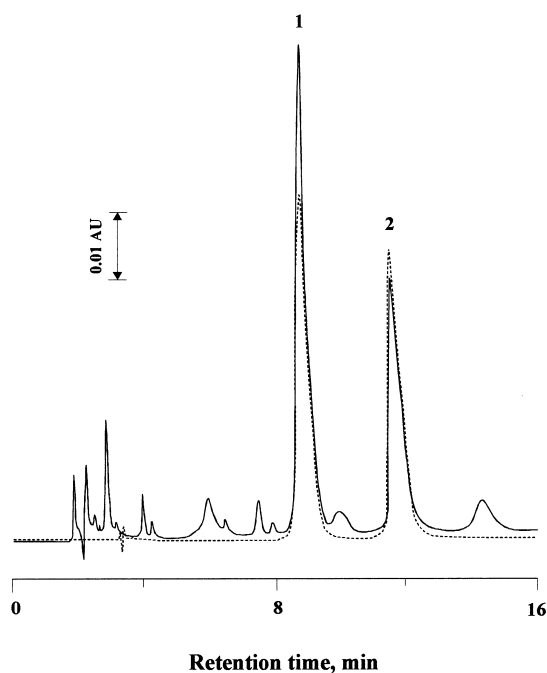


Fig. 1. HPLC chromatograms of a standard solution and of apple juice containing patulin and 5-HMF; (1) 5-HMF, (2) patulin.

Commercial apple juices were produced by blending different apple juice concentrates.

From a public health viewpoint, our results do not compare favorably with most studies reported from other countries in recent years. A German survey reported that six out of 28 apple juice samples tested contained patulin (21%) at trace levels [9]. A survey conducted during 1980 showed that patulin was detected in 70% of samples tested (total number of samples was twenty) at concentrations ranging from 1 to 38  $\mu\text{g}/\text{l}$  [10]. In 1992, an Australian (New South Wales) survey reported that 50% of sixteen apple juice concentrate samples tested contained patulin, at a maximum concentration of 646  $\mu\text{g}/\text{l}$  [6]. A New Zealand survey reported that 15% of apple juices sampled contained patulin levels ranging from 106 to 216  $\mu\text{g}/\text{l}$  [11].

Our results indicated varying ranges of patulin contamination between processors. Patulin contamination in apple juice concentrates produced by three companies are presented in Table 1. The highest patulin contamination was found in the products of company A. This result can be attributed to the

different sources of apple fruits and their quality, as well as the different processing conditions used by the companies. It is thought that the location of the processing plant of company A also played an important role.

The majority of products made by company A contained more than 50 µg/l of patulin (43 samples), and the maximum level found was 376 µg/l. Patulin contamination in the products made by company A varied between batches and ranged from 18 to 376 µg/l. None of the products of the three companies were found to be free of patulin.

It can be concluded from the results of this study that the incidence of patulin in apple juice concentrates produced in Turkey is high, with a significant proportion of the products exceeding the 50 µg/l limit for apple juice set by the WHO and certain European countries. It should be pointed out that the method used for determining the concentrations of patulin in apple juice concentrates was found to be very useful in terms of ease of operation, speed, economy and sensitivity.

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