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Full Length Research Paper

High performance liquid chromatographic determination of patulin in apple juice: Investigation of its contamination levels in Saudi Arabia

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A simple method is used for the determination of patulin in apple juice. The samples were extracted with ethyl acetate and the extract was cleaned up by using sodium carbonate solution. Patulin was then determined reverse phase high performance liquid chromatography (HPLC) using a 25 cm Zobrax XDB C_{18} column and a variable wavelength detector set at 276 nm. Water-Acetonitrile (4:96) was used as the mobile phase at a flow rate of 1 ml/min. The occurrence of patulin as a toxic mycotoxin contamination was investigated in 120 samples of apple Juice commercially available in Saudi Arabia by using an HPLC method with a concentrations ranges from 0.057 to 0.104 µg/ml. The primary aim of this investigation was to determine the amount of patulin content in apple juice and pay attention that it may cause health problems to the consumer.

Key words: Mycotoxins, patulin, apple juice, HPLC.

INTRODUCTION

Mycotoxins are toxic secondary metabolites produced by molds and can contaminate a wide variety of food and feeds. However, one fruit mycotoxin, patulin, has received a fair amount of attention. It was first isolated in 1940's but it is now known to occur world-wide in apple and apple products (Weigert et al., 1997; Trucksess and Tang, 1999; Pienontese et al., 2005; González-Osnaya, 2007).

Patulin (4- hydroxy - 4H furo [3.2-C] pyran-2 (6H)-one) Figure 1 is considered to be a mycotoxin produced by several fungal species of the gevera *Penicillium*, Aspargillus and *Bryssochamys* (IARC, 1986).

Levels of patulin in fruit products can be reduced by removing rotten tissue from the fruit.

Patulin can therefore be used as an indicator of the quality of processed apple juice and fruit products, since appreciable concentrations of the toxin remains in the food after processing (Burda, 1992). The presence of high amounts of patulin indicates that moldy apples were used in the production of the juices. For that reason the problem of detecting low level of patulin in apple juices continues to receive attention (Weigert et al., 1997). Because of patulin toxicity, health authorities in many countries regard patulin contamination of foods as problems and have set a maximum permitted concen-tration (MPC) of 50 µg /L for patulin is apple juice appropriately diluted for consumption (Forbito and Babsky, 1985; Burda, 1992; Prieta et al., 1993). De Champdore et al. (2007) reported that human exposure to patulin can lead to serious health problem, and according to a long term investigation in rats, the World Health Organization (WHO) has set a tolerable weekly in take of 7 ppb body weight. Laboratory tests indicated a harmful effect of long exposure to patulin on the liver of mice (Gashlan, 2008). Another study suggested that Patulin causes DNA damage leading to cell cycle arrest and apoptosis in skin of mice (Saxena et al., 2008).

Since apple juice is one of the popular beverages in Saudi Arabia, this study was performed on some comercial apple juice production for monitoring of the quality of the juices by measuring the level of patulin in different types of apple juice.

Many methods have been developed for measuring patulin in apple juice. These include methods based on thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) (Fritz and Engst, 1981; Moller and Joseffson, 1980). The use of HPLC methods leads to an improvement known as sensitivity (Prieta et al., 1993;

Table 1. Patulin levels found in the samples of apple juice provided from six Saudi companies.

Juice samples	J1	J2	J3	J4	J5	J6
Patulin* Values	0.057±0.002	0.052±0.004	0.061±0.006	0.047±0.007	0.085±0.011	0.104±0.006

*Values are expressed as mean of samples ± standard error (µg/ml) using Student's test.

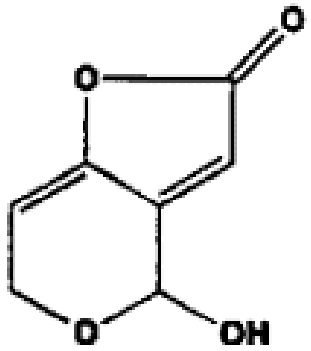

Figure 1. Structure of patulin.

Gokmen and Acar, 1996).

MATERIALS AND METHOD

Samples

Commercial apple juice samples were obtained from local markets in Jeddah. Apple juice samples were provided from six different companies

Apparatus

High-performance liquid chromatography (HPLC) Agilent Zobrax.

Column conditions

The analytical column Zobrax XDB C_{18} (25cm×4.6,5µ.) Agilent, USA was made of stainless steel and packed with 5 µm C_{18} stationary phase and operated at 40°C.

Mobile phase

Water/ Acetonitrile (4:96) were used at a flow rate of 1 ml/min.

Chemicals

Ethyl acetate (extra pure) Fisher Scientific USA, Acetonitrile Aldrich USA (HPLC grade), Sodium Carbonate (reagent grade) and unhydrous Sodium Sulfate (regent grade) were obtained from fisher and Aldrich (USA). Water used in the experiments was distilled and deionized. A standard solution of patulin was prepared by dissolving of pure patulin (Sigma) 1 mg/ml ethanol.

Extraction procedure

A 5 ml volume of single strength apple juice was extracted twice with 10 ml of ethyl acetate vigorously for 1 min using a vortex mixer. The organic phases were combined and extracted with 2 ml of 1.5% sodium carbonate solution by shaking for 1 min. The phases were allowed to separate and the aqueous phase was immediately extracted with 5 ml of ethyl acetate by shaking for 1 min. The combined organic phases were dried over 2.5 g of anhydrous sodium sulfate. Subsequently, the tried extract was filtered through a filter-paper to removing the remaining particles of anhydrous sodium sulfate. 2 ml of ethyl acetate was added to wash the filter cake layer and the filtrate obtained was combined with the filtered extract. The extract was determined by HPLC with UV detection. 10µl of this solution was injected into the column (Gokmen and Acar, 1996).

Patulin analysis

Patulin level was determined by comparing peak areas with those obtained for authentic Patulin analytical standard (Sigma chemicals. USA). The concentration of the standard was assessed using calibration at λ=276 nm against a solvent blank.

RESULTS AND DISCUSSION

The mobile phase and column used in the HPLC determination were selected to yield the maximum separation of Patulin from interfering substances in apple juice, especially 5-hydroxymethylfurfural (5-HMF). The detection wavelength was set at 276 nm, corresponding to the maximum UV absorption, as used by Fritz and Engst (1981); Gokmen and Acar (1996); Arranz et al. (2005). Figure 2 shows a chromatogram of patulin standard (Sigma) on Zobrax XDB C_{18} column using water acetonitrile (4:96,v/v) as a mobile phase at a flow-rate 1.0 ml/min. Apple juice from six Saudi producers, 120 samples in all, were analyzed as described.

Patulin level in the juice samples ranged from 0.047-0.104 µg/ml. A wide variation in the results between the samples was observed. Figure 3 shows a chromatogram of one of the commercial apple juice contaminated with Patulin. The results of the analyses of the apple juice samples are presented in Table1. Data were calculated for each sample in µg/ml. Figure 4 shows a histogram of Patulin values found in some of the apple juice samples in µg/ml. These values were shown a significant variation of Patulin level. These data indicate that the mean Patulin levels in some of the apple juices were higher than the recommended (50 µg/L) by WHO as a safety margin.

The presence of high amount of Patulin indicates that moldy apples were used in the production of juices. The

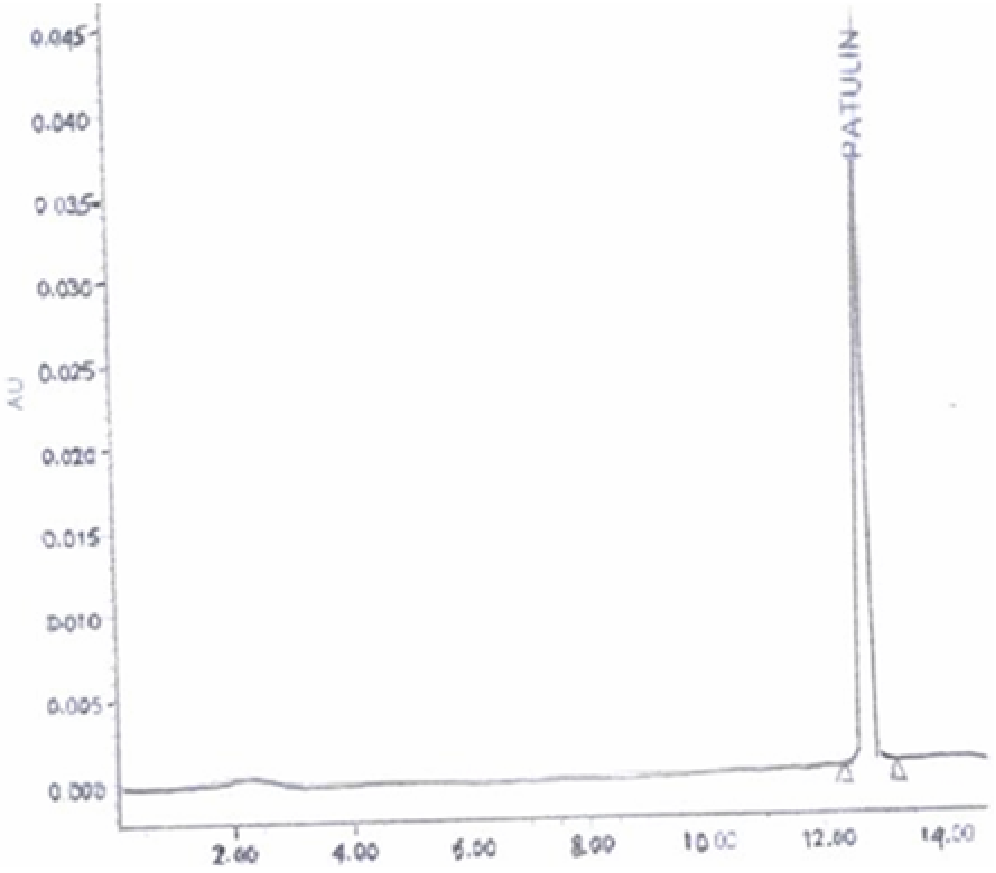

Figure 2. Chromatograme of patulin standard (Sigma).

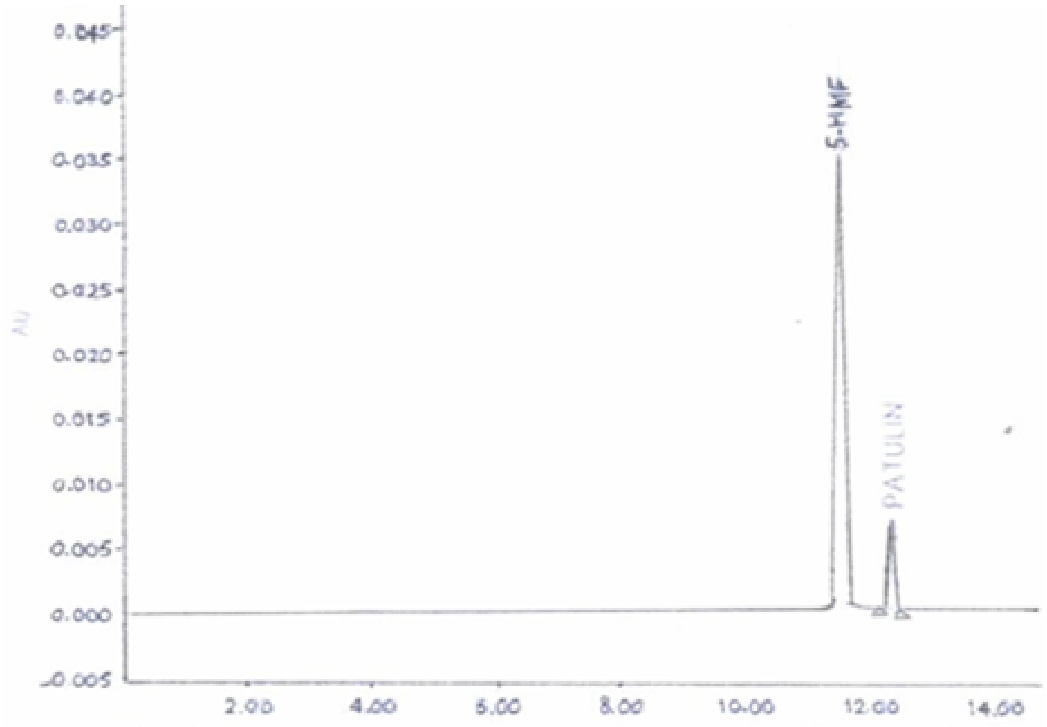

Figure 3. Chromatograme of one of a commercial apple juice contaminated with patulin.

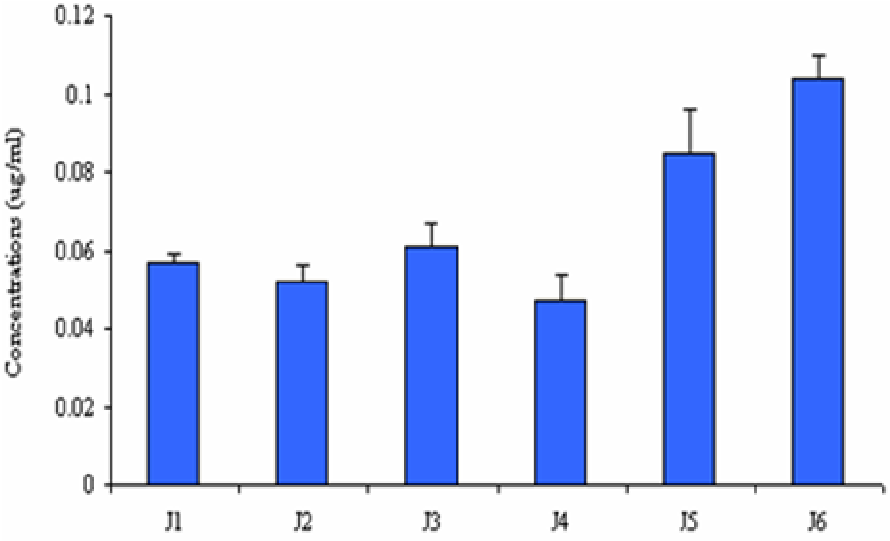

Figure 4. Histogram of Patulin values found in some of the apple juice samples in µg/ml.

results of this study suggest that Patulin found in apple juices in such high levels may cause health problems. However, Patulin has also become a quality indicator of fruit used in the processing of apple juice.

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