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PATULIN IN APPLE FRUITS:
I. SURVEY OF PATULIN IN DIFFERENT EGYPTIAN APPLE VARIETIES

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ABSTRACT

Patulin is a secondary metabolite produced mainly in rotten parts of apples by a wide range of fungi. Due to its mutagenic, teratogenic nature and possible health risks to consumers, many countries have regulations to reduce the level of patulin in apple products to become as low as practically possible. A survey of the presence of patulin was conducted during 2008 in Egyptian apple varieties (Malus domestica) using 150 apple fruits of Anna , Dorsett Golden and Golden Delicious using HPLC method.

The obtained results indicate that 30 out from 50 (60%) Anna apple fruits were positive to patulin with a concentration ranging from 1.5 to 155 ppb ,while in Dorsett Golden 20 out from 50 (40 %) fruits with a concentration ranging from 0.6 to 62 ppb , and 18 out from 50 (36 %) were positive , ranging from 1.2 to 70 ppb in Golden Delicious.

Keywords: Patulin, secondary metabolite and apple

INTRODUCTION

Patulin (g-lactone a-b insaturated {4-hydroxy-4H-furol [3,2- c]pyran-2(6H)-one}) is a secondary metabolite naturally produced by a variety of fungi like Aspergillus, Penicillium and Byssochlamys. Even though patulin is frequently associated with Penicillium expansum, many other fungi having isoepoxydon dehydrogenase (IDH) gene, which is essential to the biosynthesis of this toxin, may produce the toxin (Russell and Paterson, 2006). Patulin is frequently found in apples, apple juices and other forms of apple processed products. Patulin was also detected in barley, wheat, corn, pears, peaches, other fruits and their derived processed products. (Frank, 1977; Bartolomé, 1994; Li, 2007).

Initially patulin was described as an antibiotic due to the strong activity against different gram-positive and gram-negative bacteria including Mycobacterium tuberculosis (Russell , 2004). Several researches reported the toxicity, mutagenicity and immunotoxicity of patulin, despite being classified in group 3 by, International Agency for Research on Cancer (IARC) for lacking of evidences of its carcinogenicity in experiments with animals and humans (Alves, 2000; Wu, 2005; Schumacher, 2006; Selmanoglu, 2006). One of these studies reported that the ingestion of water contaminated with patulin at a concentration of 295mg.L^{-1} during four weeks affected the gastrointestinal system in rats, causing ulcers (Speijers *et al.,*

1988). Patulin cellular infiltration in rats at doses varying from 28 to 41 mg.kg^{-1} produced, in two weeks of experiment, lesions in the gastrointestinal tissues, including degeneration, hemorrhage, and ulceration of the gastric mucosa (Mckinley *et al.,* 1982). Mahfoud (2002) found that micromolar concentrations of patulin cause a rapid and dramatic damage in human epithelial intestinal cells. Patulin is stable in low pH value of media and resistant to thermal denaturation. These properties turn its removal very difficult through pasteurization, for example (Dombrink-Kurtzman and Blackburn, 2005).Stott and Bullerman, (1975) found that apple and apple products were the major human dietary sources of patulin. Surveys have revealed frequent occurrences of patulin in apple juice (Lindroth and Niskanen 1978; Brackett and Marth 1979; Wheeler *et al.* 1987; Burda 1992; Yurdun *et al.* , 2001), leading to increased safety grading the implications of human exposure to high levels of patulin. Consequently, an action level of 50 ug/L for patulin in apple juice has been established in the United States (Anon, 2002).

The present study was carried out to determine patulin in different varieties apple fruits namely Anna, Dorsett Golden and Golden Delicious varieties purchased from different local markets in Cairo.

MATERIALS AND METHODS

Materials

One hundred and fifty fruits of apples were purchased from different local markets in Cairo governorate, Egypt, during season 2008.

Methods

Extraction of patulin

Patulin was extracted from apple tissues according to the method of Brause *et al.* (1996). Briefly, 10 g of apple discs were extracted with 20ml of methyl acetate 3 times, by grinding in a mortar with a pestle. The resulting slurries were combined, and 20 ml was removed for further processing. Two milliliters of a 1.5% sodium carbonate solution were added to the extract to remove the co-extracts that might interfere with the analysis. The methyl acetate phase was then collected and dried using 1 g anhydrous sodium sulfate. The solvent was evaporated at 40 °C under a stream of nitrogen, and the residue was subsequently dissolved in 1 ml of acetic acid solution adjusted (pH value 4). The acetic acid extracts were passed through a 0.22-m Millipore syringe filter (25-mm dia, fisher scientific) before the high-performance liquid chromatography (HPLC) analysis.

Patulin assay

Patulin content was determined according the method of Brause *et al.* (1996). A Hewlett Packard 1100 HPLC (Agilent, New castle, Delaware, USA) equipped with a pda detector was used to identify and quantify patulin in apples. Ten microliters of sample was injected. Separation was conducted at room temperature on a discovery C18 column (Supelco, Bellefonte,Pa., USA) protected by a supelguard discovery c18 guide column (20 × 4 mm; supelco). The mobile phase was water/acetonitrile (90:10) (HPLC grade,

fisher scientific) at a flow rate of 1 ml/min. Patulin concentration was calculated in reference to the peak area (wavelength set at 276 nm) of patulin working standard solution(patulin =98%; Sigma-Aldrich, Milwaukee, Wis., USA). The working standard solution (100 µg/ml) was prepared in acetic acid solution (pH, 4) and serially diluted to 100, 80, 60, 40, 20, and 0 µg/ml and analyzed by HPLC, yielding a standard curve that was linear between 20 and 100 µg/ml . The standard curve was used for determination of patulin in apple fruits extracts.

RESULTS AND DISCUSSION

The results in Table (1) indicate that 60% of Anna apples varieties were positive for patulin with a concentration ranging from 1.5 to 155 ppb and 7 samples out from 50 Anna apples (14%) with a concentration more than 50 ppb. The results also indicate that 40% 0f Dorsett Golden samples were positive for patulin with a concentration ranging from 0.6 to 62 ppb and 4% had high concentration of more than 50 ppb, while in Golden Deliciou apples, positive samples were 36 % with concentration between 1.2 to 70 ppb. These results are in agreement with those of Kawashima *et al.* (2002), who reported that Patulin is frequently found in apples, pears and their juices and jams and other products derived from these fruits. Several studies have reported the contamination of patulin in apple juices in different countries, including Australia, Austria, Belgium, Brazil, Canada, France, Iran, Italy, Japan, South Africa, Spain, Sweden, United Kingdom, United States and Turkey (Spadaro, 2007).

Also in Brazil, Sylos and Rodriguez –Amaya (1999) analyzed 111 samples of processed fruit juices and 38 samples of fresh fruits. Only one juice sample was contaminated with levels of 17 $mg.L^{-1.}$ However, levels varying from 150 to 167 $mg.kg^{-1}$ of patulin were found in 14 samples of fresh fruits studied the production of patulin in cv. Gala and cv. Fuji apples inoculated with *Penicillium spp.* The authors observed production of patulin not only in the conditions of storage at room temperature, but also in refrigerated samples. Prado (2000) analyzed 13 trade brands of apple juices commercialized in Belo Horizonte, Brazil, and patulin (10 ppb) was detected in only one sample. In Parana state, Machinski and Midio (1996) analyzed samples of industrialized apple juices and out of 76 samples, 15 contained patulin in levels ranging from 6 to 77 ppb.

Patulin was also detected in other fruits, like grapes, cherries, plums, blueberries, oranges, strawberries and melons (Frank, 1977, Bartolomé, 1994 and Li, 2007). It was found in fruits that exhibited brown rot, such as bananas, pineapples, grapes, peaches, and apricots, indicating that the use of decayed fruits for processing would lead to the presence of the toxin in the products (Frank, 1977). Fungal growth and the production of patulin are common in damaged fruit. However, patulin can also be detected in visually sound fruit.

From this survey, it could be concluded that patulin was found in different concentrations in Egyptian apple fruits and we should check in fruits before processing.

Table (1): A summary of patulin concentrations in different apple fruits varieties

Apple varieties	Number of samples in the range (µg / kg)						
	No. of samples	Not detectable	0.5 less than 10	10 -34	35-50	greater than 50	Concentrations of patulin greater than 50 µg / kg
Anna	50	20	12	5	6	7	52, 53, 55, 70, 77,105 and155
Dorsett Golden	50	30	5	4	9	2	53 and 62
Golden Delicious	50	32	4	4	7	3	51 , 60 and 70

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الباتيولين في ثمار التفاح:
١. مسح لسموم الباتيولين لثمار التفاح المجمعة من اسواق القاهرة

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يعتبر الباتيولين احد نواتج التمثيل من بعض الفطريات وخاصة فى الاجزاء الفاسدة او التالفة فى التفاح. والباتيولين قادر على احداث تغيرات وراثية وجنينية ذات خطورة كبيرة على المستهلك. وكثير من البلاد تحدد التركيز المسموح به الى اقل حد ممكن عمليا . لذلك تهدف هذة الدراسة الى عمل مسح لانواع التفاح المشتراة من اسواق القاهرة المختلفة خلال عام (٢٠٠٨) لسموم الباتيولين , حيث تم تجميع وتحليل ١٥٠ عينة تفاح من اصناف *Anna* و *Dorsett Golden* و *Golden Delicious* . تم تقدير الباتيولين باستخدام جهاز HPLC . وقد اثبتت النتائج تواجد الباتيولين بنسبة ٦٠ % من ٥٠ عينة من تفاح *Anna* وكان التركيز متراوحا بين ٥و١ الى ١٥٥ جزء فى البليون و ٤٠% من ٥٠ عينة من تفاح *Dorsett Golden* وكان التركيز ما بين ٦و. الى ٦٢ جزء فى البليون وكذلك ٣٦ % من تفاح *Golden Delicious* وكان التركيز من ٢و١ الى ٧٠ جزء فى البليون . وقد أثبتت النتائج تواجد الباتيولين فى ثمار التفاح المنزرعة فى مصر .