Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 12, pp.23508-23511, December, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PATULIN CONTAMINATION IN PURE AND MIXED APPLE PRODUCTS MARKETED IN MYSORE CITY (INDIA)

Jayashree, G. V. and *FarhathKhanum

Biochemistry and Nanosciences Discipline, Defence Food Research Laboratory, Siddhartha Nagar, Mysore, Karnataka-570 011, India

ARTICLE INFO

Article History:
Received 25th September, 2015
Received in revised form
27th October, 2015
Accepted 19th November, 2015
Published online 21st December, 2015

Key words:

Patulin, Mycotoxins,
Mysore, Apple Juice,
HPLC.

ABSTRACT

Patulin is one of the most evasively considered yet extensively studied mycotoxins in apple juice. This survey on the presence of the mycotoxin-patulin, was conducted from 2013-2014 on commercial apple products which were purchased from the supermarkets and retail shops in Mysore. In this study, a total of 42 samples of apple products were analysed for patulin content, including apple juices, apple-based baby foods, apple-based jam, and mixed fruit juices. 38 samples had patulin below detectable limits. Total 4 samples were higher than 50µg/L that had patulin concentrations ranging between 85.32-877µg/l respectively. The results of this study indicate apple juices, apple-based baby foods and mixed fruit juice had shown less detectable patulin contamination. The low incidence of patulin in Mysore city commercial apple products indicate that the quality control of the industry highly standards.

***Citation*:** Jayashree, G. V. and Farhath Khanum, 2015. "Patulin contamination in pure and mixed apple products marketed in mysore city (India)", *International Journal of Current Research,* 7, (12), 23508-23511.

INTRODUCTION

Food safety has been a great concern because more and more chemicals are being exploited for promoting growth of crops, preservation, increasing the yield etc. Food is an important route of exposure to contaminants as pesticides, dioxins, heavy metals, drugs, polycyclic aromatic hydrocarbons, hormones and mycotoxins. Mycotoxins are a class of highly toxic compounds; these are the secondary metabolites produced under fastidious environmental conditions by certain molds or fungi contaminating many food stuffs (Barreira, Alvito, & Almeida, 2010). Patulin a fungal toxin was firstly proposed for therapeutic purpose because of its antibiotic properties. However, in 1960s it was reclassified as mycotoxin due to its toxicity (Puel, Galtier & Oswald, 2010). Mycotoxin- Patulin (4-hydroxy-4H-furo- [3,2-c] pyran-2(6H)-one) is a polyketide lactone. All mycotoxins are small-molecules which are mainly produced by certain fungal species of Penicillium (*Penicillium expansum*), Aspergillus (*Aspergillusclavatus*) and Byssochlamys, *Penicillium expansum* is the major producer of patulin. (Weidenbörner, 2001; Ritieni, 2003). The fruit pathogen is generally associated with damaged fruit or fruit already infected by other microorganisms in orchard or in postharvest conditions (Snowdon, 2001). These fungi grow on fruits and vegetables,

Corresponding author: Farhath Khanum,
Biochemistry and Nanosciences Discipline, Defence Food Research Laboratory, Siddhartha Nagar, Mysore, Karnataka-570 011, India.

However rotten apples and apple-based products are considered the major source of this fungal toxin, and are excellent substrates for *Penicillium expansum*. The most important factors that influence the presence of mycotoxins in crops or raw materials are the insect attack, damage to vegetables during harvest and the temperature and humidity during storage (Reddy *et al*., 2010). Patulin has also been identified occasionally in oranges, peaches, apricots, tomatoes, pears, grapes, strawberries, blueberries and their by-products (Gokmen, & Acar, 1998; Majerus & Kapp, 2002).

The principal risk arises when unfit fruit is used for the production of juices and other processed products, the causal agent of ''blue mould rot", to produce the patulin. From experiments, it has been demonstrated that the patulin is eliminated during fermentation in wines, its content decreased only by about 20% during the usual technological process of fruit juice production (Harrison, 1989). Therefore, other technological process with higher reducing levels have been employed like clarification, pressing followed by centrifugation and filtration, fining and enzyme treatment (Bissessur, Permaul & Odhav, 2001). Patulin contamination of apple juice is an effective indicator of unsound rotted apples in juice manufacture. Removal of decayed and damaged fruit or trimming of mouldy portions can significantly reduce patulin levels in apple products (Lovett, Thompson & Boutin, 1975).

This mycotoxin is easily transferred into the fruit products during the processing owing to its solubility in water. It is very stable to heat in acidic medium as in fruit juice (Anderson de Souza, Rosenthal & Rodriguez de Massaguer, 2008; Gokmen, Artik, Acar, Kahraman & Poyrazoglu, 2001).

Acute and short-term *in vivo* studies of patulin indicated gastrointestinal effects as distension, ulceration and haemorrhage, agitation, convulsions, edema, intestinal inflammation and vomiting (Speijers, 2004). Recent studies have also demonstrated that patulin alters the intestinal barrier function. In chronic studies in rats, patulin cause neurotoxicity, immunotoxicity and genotoxicity. Reproductive and teratogenicity, *in vivo* studies showed that patulin is embriotoxic (Wouters & Speijers, 1996; Moakeetal, 2005).

The maximum permitted level of patulin in fruit juices and nectars, in particular apple juices and apple juice ingredients in other beverages marketed in Europe is 50µg/kg (European Commission, 2003). The permitted threshold is lower for apples juices labelled and sold as intended for infants and young children (10µg/kg). Several studies have demonstrated the contamination of patulin in apple juices of different countries, including Australia, Austria, Belgium, Brazil, Canada, France, Iran, Italy, Japan, South Africa, Spain, Sweden, United Kingdom, United States, Turkey (De Sylos & Rodriguez-Amaya, 1999; Moake, Padilla-Zakour & Worobo, 2005).

Liquid-Liquid extraction and Solid-phase extraction (SPE) are the traditional methods of sample preparation in the analysis of Patulin in food samples prior to the analysis by Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS) and Liquid Chromatography-Mass Spectrometry (LC-MS) (Elvira, Gaspar, & Lucena, 2009; Iha, de Souza & Sabino, 2009; Kataoka, Itano, Ishizaki, & Saito, 2009; Moukas, Panagiotopoulou & Markaki, 2008; Songsermsakul, & Razzazi-Fazeli, 2008). Among them, HPLC coupled with UV detection, has been found to be the most suitable in Patulin trace analysis as it exhibits strong UV absorption at 276 nm (Al-Hazmi, 2010; Funes & Resnik, 2009; Li, Wu, Hu & Wang, 2007).

The aim of this study was to determine the patulin levels in some apple-based- juices, largely consumed by the population and to compare the levels of patulin contamination to that of WHO. The World Health Organization recommends a maximum concentration of 50µg/L in apple juice ("Food borne hazards, World Health Organization". Retrieved 2007-01-22). To execute this study, we validated a method for a quantitative analysis of patulin by using High-Performance Liquid Chromatography (HPLC) method coupled with UV detection.This method has better precision and sensitivity and it focused mainly on extraction. There are no reports so far on the patulin contamination of the fruit juices sold in Mysore.

MATERIALS AND METHODS

Chemicals and reagents

Patulin standard was procured from Sigma–Aldrich; sodium carbonate was purchased from Merck and acetic acid from analytical grade. Ethyl acetate was obtained from analytical grade and Acetonitrile (HPLC grade). Acidified water was prepared adjusting the pH to 4 with acetic acid (analytical grade).

Preparation of Patulin working solution

Patulin stock solution (10mg/1ml) was prepared in DMSO and stored at-20˚C. Working solution was prepared from this stock (0.5mg/ml).

Samples

A total of 42 apple-based products were analyzed for patulin content, including apple juices (14), apple-based baby foods (18), apple-based jam (3), and also from mixed fruit juice (7). They were purchased in Mysore supermarkets and retail shops during April 2013 to September 2014.All samples were stored in their original packages at room temperature until analysis. The samples were opened and thoroughly homogenised.One aliquot was processed and four aliquots were stored at-20˚C for triplicate analysis.

Preparation of samples and patulin analysis

The sample preparation was performed according to the method of Moukas, Panagiotopoulou, and Markaki (2008) and analysed using HPLC with UV detector. A portion of 10g of sample was transferred to a 50 ml centrifuge tube. 25 ml of Ethyl acetate was added and the tube was shaken vigorously for 3 min using a mixer. The mixture was centrifuged at 4500 rpm at 25˚C for 5 min. The organic upper layer was transferred to another centrifuge tube and the aqueous phase was re-extracted twice with 20 ml of ethyl acetate. The organic layers were combined and 2 ml of 2% Na_2CO_3 solution was added and shaken the tube vigorously for three times. Five millilitre(ml) of ethyl acetate was added to Na_2CO_3 solution and shaken the tube vigorously for 3 min using mixer. The pH value of the solution was adjusted to 4 with 36% acetic acid. The solution was evaporated to dryness, and 1 ml of acetonitrile solution (5%) was dissolved to the residue. All samples were filtered through a 0.22 µm syringe filter (Millipore) prior to analysis with HPLC-UV.

Chromatography Conditions

Patulin contamination were identified using a UV detector (JASCO Inc., Easton, MD,USA) equipped with RPC_{18} column (JASCO Inc.), 4.5mm diameter, 250mm length, 5micron particle size, (pump model 1850)soft ware LC.Net. The composition of mobile phase was acetonitrile: water (5:95, v/v). The volume of injection was 20µl, the flow rate of the mobile phase was 1.5 ml/min and wavelength of detection was 276 nm. The apple juices were identified by comparing the retention time (RT) of the unknowns with the standard.

RESULTS AND DISCUSSION

Patulin is a mycotoxin secreted mainly by fungi of the penicillum species. Exposure generally results from consumption of apple based fruit juices.

Table 1. Occurrence of patulin in commercial fruit juices collected from the Mysore supermarkets and retail shops

Sample	No	Contaminated	RT (mins)	PAT content in positive samples (µg/l)
Apple Juices	14	2	9.400	336-693
Apple-Based Baby Foods	18	1	9.447	85.32
Apple-Based Jam	3	0	0	0
Mixed Fruit Juice	7	1	9.447	877.22
Total	42	4	Std-9.480	85.32-877

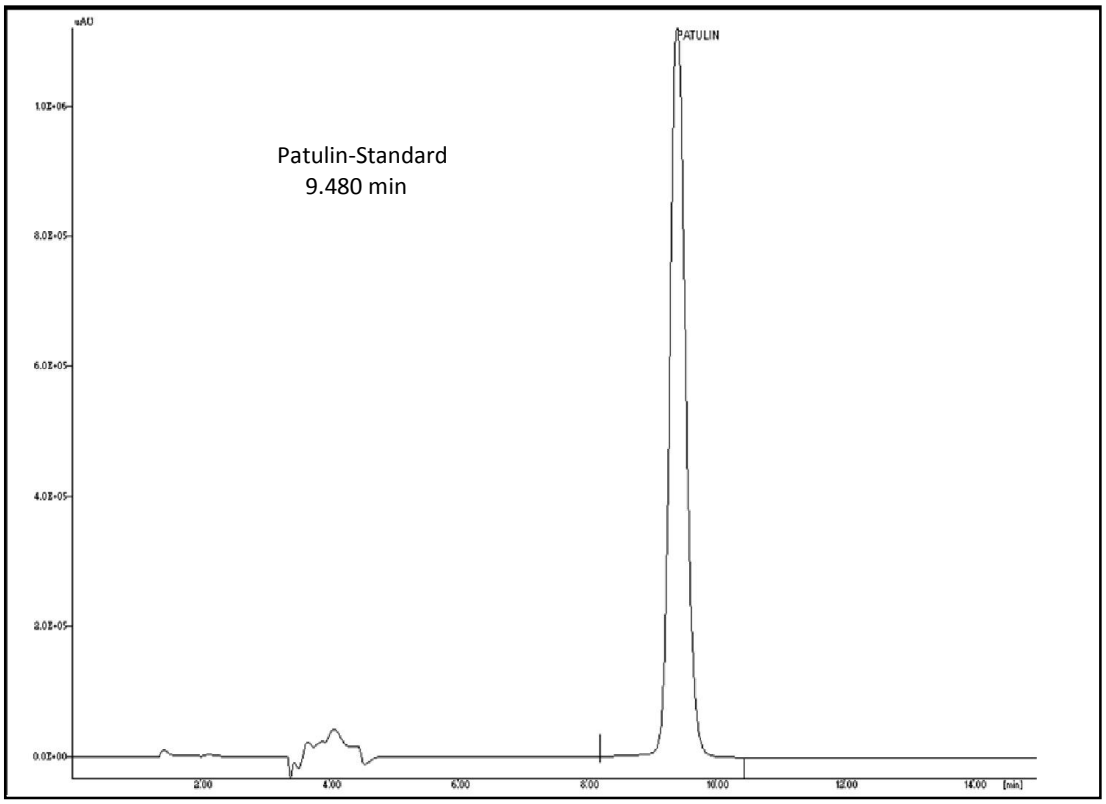

Figure 1. Patulin standard

An attempt was made to detect fungal contamination in the apple juice. In this study, a total of 42 samples of apple products were analyzed for patulin content, Total 4 samples were higher than 50µg/l as shown in the Table 1, patulin standard as shown in Figure 1. Chromatograms obtained showed that patulin has a retention time (RT) nearly 9.480 ± 0.5. Patulin production occurs from postharvest other than that it can also take place during harvest time. Organic farming practices could favours insect damage and fungi infections. The physical damage provides an access for patulin-producing fungi into the fruit, and thus the majority of infections by *Penicillium expansum*in apples are due to injuries caused by insects within apples (Moake, Padilla-Zakour & Worobo, 2005) and also factors that could influence the patulin occurrence in apple juice are the type of agriculture (Piemontese, Solfrizzo & Visconti, 2005).

Control of patulin levels in apple juice is attainable in practice. (Lovett Thompson & Boutin, 1975) purposefully contaminated apples in a controlled manner with patulin producing mold, and then successfully reduced patulin contamination to approximately 90% by trimming away the rotten portion of the fruit. FDA believes that control by processors of patulin levels to 50µg/kg or below can be achieved principally by removing spoiled and visually damaged apples from the product stream used for the production of apple juice. Other measures such as water treatment also may be effective in reducing patulin levels. Sydenham *et al*. (1995) found a significant reduction of patulin levels following an initial water treatment step and removal by hand of rotten and damaged fruit prior to juice production.

Conclusion

In the present study we identified patulin contamination in the apple based products out of 42 samples analysed patulin was detected in 4 samples. The apple juices, apple-based baby foods and mixed fruit juice showed less detectable of patulin contamination. The low incidence of patulin in Mysore commercial apple products indicates that the fruit and the processing practices, utilize are of good standards. Surveys have not been conducted in Mysore in order to scrutinize patulin levels in apple-based products and to resolve the associated risk for human health.

Acknowledgement

The authors are thankful to Dr. Harsh Vardhan Batra, Director, Defence Food Research Laboratory, Mysore, for providing all the necessary facilities, constant guidance and encouragement during this investigation.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

"Food borne hazards, *World Health Organization*". Retrieved 2007-01-22.

Al-Hazmi, N. 2010. Determination of patulin and ochratoxinA using HPLC in applejuice samples in Saudi Arabia. *Saudi Journal of Biological Sciences*, 17, 353e359.

Anderson de Souza, S., Rosenthal, A. and Rodriguez de Massaguer, P. 2008. The fate of patulin in apple juice processing: a review. *Food Research International*, 41,441e453.

Barreira, M., Alvito, P. and Almeida, C. 2010. Occurrence of patulin in apple-basedfoodsin Portugal. *Food Chemistry,* 121, 653e658.

Bissessur, J., Permaul, K. and Odhav, B. 2001. Reduction of patulin during apple juice clarification. *Journal of Food Protection*, 64(8), 1216–1219.

De Sylos, C. M. and Rodriguez-Amaya, D. B. 1999. Incidence of patulin in fruits and fruit juices marketed in Campinas, Brazil. *Food Additives and Contaminants*, 16(2), 71 74.

Elvira, M., Gaspar, M. and Lucena, A. 2009. Improved HPLC methodology for food control-furfurals and patulin as markers of quality. *Food Chemistry*, 114, 1576e1582.

European Commission 2003. Regulation N. 1425/2003 of 11 August 2003 amending Regulation N. 466/2001 as regards patulin. Official *Journal of the European Union* from 12.08.2003 L 203/3.

Funes, G. J. and Resnik, S. L. 2009. Determination of patulin in solid and semisolidapple and pear products marketed in Argentina. *Food Control*, 20, 277e280.

Gokmen, V. and Acar, J. 1998. Incidence of patulin in apple juice concentratesproduced in Turkey. *Journal of Chromatography A,* 815, 99e102.

Gokmen, V., Artik, N., Acar, J., Kahraman, N. and Poyrazoglu, Z. 2001. Effects of various clarification treatments on patulin, phenolic compound and organic acid compositions of apple juice. *European Food Research and Technology*, 213, 194e199.

Harrison, M. 1989. Presence and stability of patulin in apple products – A review. *Journal of Food Safety*, 9(3), 147–153.

Iha, M., de Souza, V. C. and Sabino, M. 2009. Single-laboratory validation of a liquid chromatography method for the determination of patulin in apple juice. *Food Control,* 20, 569e574.

Kataoka, H., Itano, M., Ishizaki, A. and Saito, K. 2009. Determination of patulin in fruit juice and dried fruit samples by in-tube solid-phase microextraction coupled with liquid chromatographyemass spectrometry. *Journal of Chromatography* A, 1216, 3746e3750.

Li, J., Wu, R., Hu, Q. and Wang, J. 2007. Solid-phase extraction and HPLC determinationof patulin in apple juice concentrate. *Food Control*, 18, 530e534.

Lovett, J., Thompson, R. G. and Boutin, B. K. 1975. Trimming as a meansof removing patulin from fungus rotten apples. *Journal of AOAC International*, 58, 909–911.

Lovett, J., Thompson, R.G. and Boutin, B. 1975. Trimming as a means of removing patulin from Fungus rotted apples. *J AssocOff Anal Chem* Sep;58 (5): 90911.

Magkos, F., Arvaniti, F. and Zampelas, A. 2006. Organic food: buying more safety or just peace of mind? a critical review of the literature. Crit. Rev. *Food Sci. Nutr*. 46, 23–56.

Majerus, P. and Kapp, K. 2002. Reports on tasks for scientific cooperation, task 3.2.8. Assessment of dietary intake of patulin by the population of EU Member States. SCOOP Report. Brussels, Belgium:European Commission. http://europa.eu.int/comm/food/fs/scoop /3.2.8_en.pdf.

Moake, M.M., Padilla-Zakour, O.I. and Worobo, R.W. 2005. Comprehensive reviewof patulin control methods in foods. Compr. *Rev. Food Sci. Food Saf*. 4, 8–21.

Moake, M.M., Padilla-Zakour, O.I. and Worobo, R.W. 2005. Comprehensive review of patulin control methods in foods. Compr.Rev. *Food Sci. Food Saf*., 4, 8–21.

Moukas, A., Panagiotopoulou, V. and Markaki, P. 2008. Determination of patulin in fruit juices using HPLC-DAD and GC-MSD techniques. *Food Chemistry,* 109, 860e867.

Moukas, A., Panagiotopoulou, V. and Markaki, P. 2008. Determination of patulin infruit juices using HPLC-DAD and GC-MSD techniques. *Food Chemistry*, 109, 860–867.

Piemontese, L., Solfrizzo, M. and Visconti, A. 2005. Occurrence of patulin in conventional and organic fruit products in Italy and subsequent exposure assessment. *Food Addit.Contam.* 22, 437–442.

Puel, O., Galtier, P. and Oswald, I.P. 2010. Biosynthesis and toxicological effects of patulin. *Toxins 2,* 613–631.

Reddy, K.R.N., Salleh, B., Saad, B., Abbas, H.K., Abel, C.A. and Shier, W.T. 2010. An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Rev. 29*, 3–26.

Snowdon, A. L. 2001. Color atlas of post-harvest diseases & disorders of fruits & vegetables Introduction and fruits (Vol. 1). *Ames*: Iowa State University Press.

Songsermsakul, P. and Razzazi-Fazeli, E. 2008. A review of recent trends in applications of liquid chromatographyemass spectrometry for determination of mycotoxins. *Journal ofLiquid Chromatography and Related Technologies*, 31(11e 12), 1641e1686.

Speijers, G. J. A. 2004. Patulin. In N. Magan& M. Olsen (Eds.), Mycotoxinsin food – detection and control (pp. 339–352). Cambridge, England: Woodhead Publishing Ltd.

Sydenham, E. W., Vismer, H. F., Marasas, W. F. O, Brown, N., Schlechter, M., Westhuizen, L. and Rheeder, J. P. 1995. Reduction of patulin in apple juice samples—influence of initial processing. *Food Control*, 6, 195–200.

Weidenbörner, M. 2001. Encyclopaedia of food mycotoxins. Berlin: *Springer-Verlag*.

Wouters, M. F. A. and Speijers, G. J. A. 1996. Patulin. In Food additivesseries 35. Toxicological evaluation of certain food additives and contaminants (pp. 337–402). Geneva, Switzerland: *World Health Organisation*.

****