Survey of patulin contamination in fruit juice in Thailand

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Abstract

Various kinds of fruit juice such as apple, orange, guava, passion fruit have been collected from supermarkets and drinking trolleys in Bangkok and suburb since 2003 for determining of patulin contamination. Patulin was extracted from fruit juice with ethyl acetate by liquid-liquid partition extraction, cleaned up with silica gel column and determined by HPLC-UV detector. It was found that 14 samples out of 103 samples (14 %) were contaminated with patulin in the range 4.55-35.97 µg/L. The result showed that there was not serious contamination of patulin in fruit juice especially apple juice since the maximum regulation level of patulin in apple juice is set at 50 µg/L (Regulation (EC) No 1425/2003).

Key words: patulin, apple juice, fruit juice

Introduction

Patulin is a mycotoxin that is produced by certain fungi belonging to *Penicillium*, *Aspergillus*, and *Byssoschylamys*. Patulin has been found to occur in a number of foods including apple juice, apples and pears with brown rot^{1,2)}, flour³⁾, and malt feed⁴⁾. *P. expansum* is a common spoilage microorganism in apples, and it is the major sources of patulin production in apples and apple juice made from affected fruit. Patulin is reported to be destroyed by fermentation and thus patulin is not found in either alcoholic fruit beverages or vinegars produced from fruit juices. Thermal processing appears to cause only moderate reduction in patulin levels, thus patulin present in apple juice will survive the pasteurization process⁵⁻⁷⁾.

Patulin has been found to cause a range of adverse effects in laboratory animal. It can affect the developing fetus, immune system, nervous system and gastrointestinal tract and cause DNA damage. There is concern that similar effects may occur in human. Due to potential adverse effects from long-term exposure to patulin, many countries have established an action level of 50 µg/L for patulin in apple juice⁸⁾.

Thai government initiated the Healthy Thailand policy in 2005 concern with 5 areas as exercise, diet, emotion development, disease reduction and environmental health. Health promotion and health preventive have been introduced to people in order to reduce disease. Since then people have tried to fit their health with good exercise and good foods. Usually good foods are composed of 5 groups as carbohydrate, protein, fat, vitamin and mineral. Nowadays, most of people eat outside and they think that they are lacking of vitamin and mineral from their food. So they try to eat vegetable and fruit to make them good health when they are at home. But there is not sufficient time for them to prepare such foods. A ready, made vegetable and fruit juices are their first choice. The annual forecast report showed an

increasing rate of 5 % for selling of fruit juice in 2006. The volume is estimated to be 75 million liters and the commercial value is 14,000 million baht. Among the fruit juices, apple juice is the popular one. Since patulin has been found to occur at high levels in some apple juice products offered for sale or import in many countries. In order to protect people from health hazard from patulin contamination in fruit juice, Bureau of Quality and Safety of Food was established the determination of patulin in fruit juice and analysed many kinds of fruit juice selling in the market in Bangkok and suburb.

Materials and methods

One hundred and three samples of various kinds of fruit juice such as apricot, apple, corn milk, ginkgo, orange, guava, passion fruit have been collected from supermarkets and drinking trolleys in Bangkok and suburb since 2003.

Standard patulin was dissolved with absolute ethanol to be 10 µg/ml and it was measured UV spectrum from 350-200 nm. The maximum absorbance at 275 nm was used to calculate the correct concentration of patulin. Aliquot portion of patulin standard solution was used to spike the blank sample at 50 µg/L. Another aliquot portion of patulin standard was evaporated and dissolved with 0.1 % TFA in H₂O to prepare working standard solution at concentration range 0.5-15 µg/mL for HPLC analysis.

The pool sample of apple juice from another lot was a sample first analysed for patulin by 6 different methods to see whether there was contaminated with patulin or not. The pool sample of apple juice with no patulin contamination (blank sample) was spiked with patulin at 50 µg/kg and they were analysed to see percent recovery of patulin by 6 different methods and the best method was selected and used for patulin analysis in 103 fruit juice samples mentioned above. The 6 different methods were as the following:

1. Apple juice 15 mL was extracted with ethyl acetate 15 mL in the centrifugal tube by shaking 2 min and centrifuged at 1106 g for 2 min. The upper ethyl acetate layer was collected in the Erlenmeyer flask. The lower aqueous layer was further extracted with 15 mL ethyl acetate for 2 times, the ethyl acetate layer (45 mL) was combined in the same flask and anhydrous sodium sulfate 10 g was added to the flask, mixed and shaken occasionally for 30 min. The clear ethyl acetate layer was filtered through the filter paper into the round bottom flask and it was evaporated to dryness under reduced pressure in the rotary evaporator at 50 °C. The residue was dissolved in chloroform 2 mL and anhydrous sodium sulfate 0.1 g was added in the flask, mixed and kept the flask in the dark for 1 hour. Transferred 1 mL and put into Sep-Pak silica gel column (which was conditioned with chloroform 5 mL before use), washed the column with 1 mL chloroform, 1mL chloroform: ethyl acetate (8:2, v/v) and eluted with 1 mL chloroform: ethyl acetate (1:1, v/v) and 3 mL chloroform: ethyl acetate (2:8, v/v). The eluate (4 mL) was evaporated to dryness and the residue was dissolved in 250 µL 2.5 % acetic acid in H₂O and transferred to a small vial for HPLC analysis using the following condition: injection volume 5 µL, C18 column 250 x 4.6 mm, 5 µm, mobile phase CH₃CN:0.1 % TFA (5:95, v/v), flow rate 1 mL/min, UV detector at 279 nm. The peak area of the patulin extracted from the sample was compared with the peak area of the standard patulin from the calibration curve at the range 0.5-15 µg/mL.

2. Apple juice 50 mL was extracted with H₃PO₄ 1 mL and ethyl acetate 100 mL in the separatory funnel by shaking 10 min. The upper ethyl acetate layer was collected in another separatory funnel. The lower aqueous layer was further extracted with 50 mL ethyl acetate. The ethyl acetate layer (150 mL) was combined in the same funnel and 2 % NaHCO₃ 50 mL was added to the funnel, mixed and shaken for 10 min. The upper ethyl acetate layer was collected in the Erlenmeyer flask. Anhydrous sodium sulfate 30 g was added to the flask, mixed and shaken occasionally for 30 min. The clear ethyl acetate layer was filtered through

the filter paper into the round bottom flask and it was evaporated to dryness under reduced pressure in the rotary evaporator at 50 °C. The residue was dissolved in chloroform 1 mL and the chloroform solution was put into florisil column (which was packed with florisil 0.7 g in chloroform), washed the column with 30 mL chloroform and eluted with 30 mL chloroform: MeOH (5:1, v/v). The eluate (30 mL) was evaporated to dryness and the residue was dissolved with 500 µL 2.5 % acetic acid in H₂O and transferred to a small vial for HPLC analysis as mentioned in No.1.

3. Apple juice 5 mL was extracted with ethyl acetate 10 mL in the test tube. The upper ethyl acetate layer was collected in another test tube. The lower aqueous layer was further extracted with 10 mL ethyl acetate. The ethyl acetate layer (20 mL) was combined in the same test tube. Aliquot of 5 mL was taken and put into another test tube, then 1.5 % NaHCO₃ 2 ml was added and shaken 2 min. The upper ethyl acetate layer was collected in the Erlenmeyer flask. The lower aqueous layer was further extracted with 5 mL ethyl acetate. The ethyl acetate layer (10 mL) was combined in the same flask. Anhydrous sodium sulfate 3 g was added to the flask, mixed and shaken occasionally for 30 min. The clear ethyl acetate layer was filtered through the filter paper into the round bottom flask and it was evaporated to dryness and the residue was dissolved with 500 µL 2.5 % acetic acid in H₂O and transferred to a small vial for HPLC analysis as mentioned in No.1.

4. Apple juice 50 mL was extracted with ethyl acetate 50 mL in the separatory funnel by shaking 10 min. The upper ethyl acetate layer was collected in the Erlenmeyer flask. The lower aqueous layer was further extracted with 50 mL ethyl acetate. The ethyl acetate layer (100 mL) was combined in the same flask. Anhydrous sodium sulfate 20 g was added to the flask, mixed and shaken occasionally for 30 min. The clear ethyl acetate layer was filtered through the filter paper into the round bottom flask and it was evaporated to remain about 25 mL. Then it was transferred to a cylinder and benzene was added and adjusted to be 100 mL. The 100 mL solution was put into silica gel column (which was packed with silica gel 15 g in benzene), eluted with 200 mL benzene:ethyl acetate (75:25, v/v). The eluate (200 mL) was evaporated to dryness and the residue was dissolved in 500 µL 2.5 % acetic acid in H₂O and transferred to a small vial for HPLC analysis as mentioned in No.1.

5. Performed the same procedure as No.4 but used chloroform instead of benzene.

6. Performed the same procedure as No.4 but used toluene instead of benzene.

The best method was method No.6. This method was changed a little bit in order to get better peak shape of the patulin for HPLC analysis. 0.1 % trifluoroacetic acid (TFA) was used instead of 2.5 % acetic acid. This method was used to analyse 103 samples of fruit juices as mentioned above.

Results and discussion

The first method was modified from the German expert. The second method was modified from the Japanese expert. The third method was modified from AOAC Official method 995.10 Patulin in Apple Juice Liquid Chromatographic method (2000). The fourth method was modified from AOAC Official method 974.18 Patulin in Apple Juice Thin Layer Chromatographic method (2000). The fifth and the sixth methods were established because benzene was very toxic to health and very difficult to buy from the company. The percent recovery of patulin from all of the methods were 95.96, 23.00, 126.56, 74.72, 75.60 and 21.28 % respectively. The percent recovery was not so good due to the interference of HMF [5-(hydroxymethyl)-2-furaldehyde]. HMF is a compound formed in the thermal treatment (Maillard reaction) of foods containing reducing sugars and amino acids, it is naturally found in apple juice, honey, baked food, etc. Thus aliquot portion of HMF (0.5 µg/mL) was added to patulin standard solution and the resolution of the two peaks were better when the dissolved

solution was changed from 2.5 % acetic acid to 0.1 % TFA. Though the first trial, the percent recovery of patulin was too small, the sixth method was chosen to be the best method due to the ease of the method and the facility available in the laboratory. Since the method validation of the sixth method showed the average percent recovery of 83 % over the method range 0.5-15 µg/mL with the accepted percent relative standard deviation (% RSD = 0.63–3.46). The low percent recovery at the first trial was due to incomplete dissolution of the residue in 2.5 % acetic acid.

The result of patulin analysis in 103 samples was showed in Table 1. All of the fruit juices except apple juice were not contaminated with patulin. Only apple juices were contaminated with patulin in the range 4.55-35.97 µg/L. The number of contamination was 14 samples out of 103 samples (14 %). Among the apple juices, the contamination was 14 samples out of 52 samples (27 %) and the most contaminated one was apple juice 100 % as showed in Table 2. According to the regulation from many countries which have established an action level of 50 µg/L for patulin in apple juice (Regulation (EC) No 1425/2003). The level found in the fruit juices in this study was consider safe for drink, even though Thailand did not have any regulation for patulin contamination in food and feed.

Table 1. Analytical results of patulin contamination in juice in Thailand

Juice	No. of sample	No. of positive sample	Patulin (µg/L)
Apricot	1	-	-
Asiatic pennywort	1	-	-
Apple juice	52	14	4.55-35.97
Beetroot	3	-	-
Carrot	6	-	-
Chinese vegetable black jelly	1	-	-
Coconut milk	1	-	-
Corn milk	1	-	-
Guava	4	-	-
Ginkgo	1	-	-
Orange	3	-	-
Passion fruit	3	-	-
Roselle	1	-	-
Sugarcane	4	-	-
Tea apple flavor	1	-	-
Mix	20	-	-
Total	103	14	4.55-35.97

Table 2. Classification of apple juice

Apple juice	No. of sample	No. of positive sample	Patulin (µg/L)
25 %	1	-	-
30 %	5	-	-
56 %	1	-	-
100 %	38	13	4.55-35.97
100 % (sparkling)	6	-	-
concentrate	1	1	9.83
Total	52	14 (27 %)	4.55-35.97

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