Incidence of patulin contamination in apple juice produced in Iran

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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 and teratogenic nature and possible health risks to consumers, many countries have regulations to reduce level of patulin in apple products to as low as practically possible. A survey of the presence of patulin was conducted in 2002 on 65 locally produced commercial apple juice and apple juice concentrates purchased from retail outlets or producers in Iran. In this study 42 samples of apple juice and 23 apple juice concentrate surveyed for patulin contamination. Results of the study showed in 69% of apple juice and 78% of the apple juice concentrates level of patulin found was higher than 15 μg/l. Overall 33% of the apple juice samples had patulin levels higher than 50 μg/l with maximum level of 285 μg/l and 56% of the apple juice concentrates with patulin level of higher than 50 μg/l and maximum level of 148 μg/l. Although the mean concentration of patulin in apple juice samples surveyed was slightly lower than maximum level recommended by the Codex Alimentarius, fairly high incidence of patulin in these samples indicates the need for improving production techniques by industry in order to reduce the incidence and level of patulin contamination in apple juices.

Keywords: Patulin; Apple juice; Mycotoxin

1. Introduction

Patulin is a member of compounds known as mycotoxins. It is a secondary metabolite produced by a wide range of Aspergillus and Penicillium fungi. Patulin has mainly been found in apples and apple products and, occasionally in other fruits such as pears, apricots, peaches and grapes and it is mainly produced in rotten parts of the fruits.

Patulin is highly toxic following acute exposure. Short-term and subchronic exposure to patulin causes teratogenic effect. However, lack of mutagenic activity by patulin in short-term mutagenic assay systems has been noted (Kubacki, 1986). Several countries regulate patulin levels in fruit juices and other fruit products. At present Codex Alimentarius recommends that level of patulin in fruit juice and fruit products should be lower than 50 μg/l (FAO, 1997; WHO, 1995). The control of patulin in fruit juice and fruit products could be achieved by using healthy fruit, hygienic storage, sorting damaged and rotten fruits, trimming off rotten tissue, filtration through activated charcoal, pasteurization and addition of sulfur dioxide or ascorbate (BSDA, 2001).

Several surveys on level of patulin contamination have been conducted in some countries. Twenty-three percent of 328 fruit product samples surveyed between 1989 and 1990 in UK were contaminated with patulin and 22% of these samples contained patulin levels between 51 and 1130 μg/l (Burda, 1992). Among 100 apple juice samples surveyed for incidence of patulin in Spain, 82 samples were contaminated with a mean of 13.8 μg/l and seven of these samples had patulin levels above 50 μg/l (Prieta, Moreno, Diaz, Suarez, & Dominguez, 1994).
All of the 215 samples of apple juice concentrates were analyzed in Turkey for patulin in 1994 were contaminated ranging from 7 to 376 µg/l and 98 of these samples contained patulin level above 50 µg/l (Gokmen & Acar, 1998). In Brazil 111 samples of processed fruit juices and 38 samples of sound fruits were analyzed for patulin. Only one out of 30 samples of apple juice was found positive at 17 µg/l. However, 150–267 µg/kg of patulin was found in 14 spoiled apple samples (De Sylos & Rodriguez-Amaya, 1999).

Although the mean of patulin contamination in apple juices was slightly less than 50 µg/l, 33% of samples had patulin level higher than 50 µg/l. The maximum level found was 285.3 µg/l. Contamination with patulin was substantially higher in apple juice concentrates with a mean of 61.7 µg/l and 56% of samples contained patulin higher than 50 µg/l.

### 2. Materials and methods

Sixty five samples of apple juice and apple juice concentrate randomly sampled at various retail outlets and producers in Iran between 2001 and 2002. Samples stored below 4 °C before analysis.

For analysis of the samples thin layer chromatography (TLC) method reported by the Association of Official Analytical Chemists (AOAC, 1984) has been used. However, a densitometry method using TLC scanner was used for quantification of patulin in samples. Fifty ml of fruit juice or concentrate extracted three times with 50 ml ethyl acetate. Pooled organic phase dried over 20 g anhydrous sodium sulphate and evaporated to about 25 ml. The solution cleaned up using a silica gel column running with 200 ml benzene/ethyl acetate (25:75). Effluent was collected and evaporated under stream of nitrogen gas.

Residue was dissolved in 500 µl of chloroform and 20 µl spotted on TLC plate. Thirty microliters of working standard of 10 µg/ml of patulin was also spotted on the TLC plate. Plates were developed using toluene/ethyl acetate/formic acid 90% (50:40:10). After drying, plates have been scanned using TLC scanner (model E3, CAMAG equipped with mercury lamp) at 278 nm. Concentration of patulin was calculated after correcting for recovery which was calculated between 80% and 115%.

### 3. Results

Results from the survey of commercial apple juice/concentrate for patulin contamination are presented in Table 1.

### 4. Discussion

Patulin, an important contaminant of apple products and other fruits, is a toxic secondary metabolite produced by a wide range of Aspergillus and Penicillium fungi. Mold growth and subsequent production of patulin normally occurs mainly where the surface tissue of fruit has been damaged. High patulin levels (mean = 2335 ng/g) were recorded in the rotten fractions of apples (Sydenham et al., 1995). JECFA estimated Provisional Maximum Tolerable Daily Intake (PM TD I) of 0.43 µg/kg bw/day or 3.01 µg/kg bw/week as safe exposure level (Bolger, 2002). Numerous surveys of patulin in apples and apple products have been published in the literature over the past years. Worldwide interest in patulin contamination dramatically increased over the last few years following a series of articles published the results of several surveys conducted in different countries. In these surveys, patulin concentrations up to 497 µg/l were detected in some retail apple juices sold in UK (MAFF, 1993). Overall, about 50% of apple juice samples analyzed worldwide have been shown to contain detectable levels of patulin (Kubacki, 1986; Prieta et al., 1994). The contamination of apple juice products may sometimes be as high as 8000 µg/l in apple juices made from partly rotten apples (Brackett & Marth, 1979), and even higher in apple cider obtained from industrial premises where decayed apples were not sorted out prior to processing (Wilson & Nuovo, 1973). Apple juice concentrates produced in Turkey were analyzed for patulin in 1994. All the samples were contaminated with patulin ranging from 7 to 376 µg/l and 98 of these samples contained patulin levels above 50 µg/l (Gokmen & Acar, 1998). The results from the South African survey of 60 commercial apple products for patulin contamination showed none of the samples had patulin higher above 50 µg/l and the maximum level found was 45 µg/l (Leggott & Shephard, 2001).

### Table 1

Results of survey for patulin contaminations of apple juice and concentrates

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No.</th>
<th>Patulin concentration</th>
<th>Patulin concentration</th>
<th>Patulin concentration</th>
<th>Max. (µg/l)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;15 µg/ml</td>
<td>15–50 µg/ml</td>
<td>&gt;50 µg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apple juice</td>
<td>42</td>
<td>13 (31%)</td>
<td>15 (36%)</td>
<td>14 (33%)</td>
<td>285.3</td>
<td>48.1 ± 9.5</td>
</tr>
<tr>
<td>Apple juice</td>
<td>23</td>
<td>5 (22%)</td>
<td>5 (22%)</td>
<td>13 (56%)</td>
<td>148.8</td>
<td>61.7 ± 41.6</td>
</tr>
<tr>
<td>concentrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>18 (28%)</td>
<td>20 (31%)</td>
<td>27 (41%)</td>
<td>285.3</td>
<td>54.9 ± 25.6</td>
</tr>
</tbody>
</table>
Published surveys in 1994 reported incidence of patulin in fruit juices sampled in Spain and Austria between 43% and 85% with patulin levels of 10–185 µg/l (Pittet, 1998).

In present study 42 samples of apple juice and 23 apple juice concentrate surveyed for patulin contamination. Results of the study showed in 69% of apple juices and 78% of the apple juice concentrates level of patulin was found to be higher than 15 µg/l. Overall 33% of the apple juice samples had patulin levels higher than 50 µg/l with maximum level of 285 µg/l and 56% of the apple juice concentrate with patulin level of higher than 50 µg/l and maximum level of 148 µg/l (Table 1).

Although the mean concentration of patulin in apple juice samples surveyed was slightly lower than maximum level recommended by the Codex Alimentarius, fairly high incidence of the patulin in apple juice marketed in Iran indicates the need for awareness of the apple industry to patulin contamination in fruit and the implementation of improved techniques for the production of apple products in order to reduce the incidence and level of patulin contamination in apple juices. It was also previously reported that it is possible, even when processing apples that may be pre-disposed to fungal contamination, to reduce significantly patulin concentrations to an acceptable level. It has been reported that the mean patulin level in the non-processed fruit was 920 ng/g following an initial water treatment step. Additional removal of rotten and damaged fruit prior to further processing, significantly reduced the mean patulin level in the juice to 55 ng/g (Sydenham et al., 1995).

References


