Survey of Patulin in Apple Juice and Children's Apple Food by the Diphasic Dialysis Membrane Procedure

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A survey of patulin in 100 samples of commercial apple juice and 12 samples of apple food for children collected at stores in Madrid (Spain) during 1992 was conducted. Patulin levels were determined by a dialysis diphasic membrane procedure using reversed-phase high-pressure liquid chromatography as analytical method. The results obtained in apple juice were as follows: patulin was detected in 82% of samples; 75% of the analyzed apple juice contained less than 10 μg/L, and the maximum concentration found was 170 μg/L; children's apple food samples were free of patulin. It is suggested that patulin should be used as a quality indicator of the apples employed in the elaboration of food and drink.

Keywords: *Patulin; apple juice; children's apple food; diphasic dialysis*

INTRODUCTION

Patulin is a mycotoxin produced by several *Penicillium* and *Aspergillus* species (Steiman *et al.*, 1989). It is acutely toxic to mice and mutagenic to yeast and produces tumors in rats (Brackett and Marth, 1979).

Under laboratory conditions, patulin can be produced on different food products (fruits, grains, cheese, cured meats), but in natural situations, patulin is almost exclusively found in apples and apples products (Harrison, 1989).

Among patulin-producing fungi, *Penicillium expansum* is the most commonly encountered species. This fungus is the principal cause of apple rot, being often isolated from decaying apples (Ware *et al.*, 1974). Therefore, it is not surprising to find patulin as a common contaminant of apple products, especially apple juice, worldwide (Jelinek *et al.*, 1989). Although the contamination incidence is fairly high, the level of contamination is generally low. The amount of patulin in commercial apple juice is usually less than 10 μg/L (Jelinek *et al.*, 1989).

Because of concern for human health and the possibility of using patulin as a quality indicator in foods, at least 12 European countries have established regulatory limits for patulin in various foods, usually apple and apple products. The most common limit is 50 μg/L or kg. In addition, the World Health Organization (WHO) has established a maximum recommended concentration of 50 μg/L for patulin in apple juice (Stoloff *et al.*, 1991; van Egmond, 1989).

The aim of the present work was to investigate the occurrence of patulin in apple juice and children's apple food using three different procedures based on the diphasic dialysis membrane procedure (DDMP) developed in our laboratory (Domínguez *et al.*, 1992; Prieta *et al.*, 1992, 1993; Prieta, 1993). These procedures are described below.

MATERIALS AND METHODS

Food Samples. This study encompassed a total of 100 samples of commercial apple juices and 12 samples of commercial apple foods for childrens. The samples were bought in different local stores randomly selected in Madrid between April and December 1992. The samples were stored below 4 °C prior to analysis and were analyzed immediately upon opening.

Instrumental. Extractions were made using a controlled environment incubator shaker Model G25 (New Brunswick Scientific, Edison, NJ). The liquid chromatography was accomplished using a Rheodyne injector (Cotati, CA), a Model 510 pump, a stainless steel reversed-phase column packed with Novapak C_{18} 4 μm (150 × 3.9 mm), a Model 486 ultraviolet detector, Maxima 820 software, and a Waters System interface module (Waters, Milford, MA).

Materials and Reagents. Dialysis tubing (Visking size 20/32) was obtained from Serva (Feinbiochemical, Heidelberg, Germany), and the cleanup was done by employing a silica SepPak cartridge (no. 51900, Waters). Reagents used were chloroform, ethyl acetate, and anhydrous sodium sulfate (all of analytical reagent grade) and water and tetrahydrofuran (THF) (HPLC grade). Patulin in the crystalline form was obtained from Sigma (St. Louis, MO).

Extraction Procedures. *1. Analysis of Patulin in Apple Juice by DDMP.* This procedure has been previously described by Prieta *et al.* (1993).

2. Analysis of Patulin in Apple Juice by Mini-DDMP. Patulin was extracted using 20 mL of ethyl acetate placed in a hydrated dialysis tube (30 cm length). Samples (10 mL of apple juice) and dialysis tube were placed in flasks and extracted by shaking (150 rpm) overnight at room temperature (20–25 °C).

The content of the dialysis tube was decanted and the aqueous layer removed. The volume of the organic extract was collected in a flask; thereafter, the extract was dried with anhydrous sodium sulfate. The total extract was transferred into a vial and evaporated to dryness under a gentle stream of air. The residue was then dissolved in 1 mL of chloroform (Prieta, 1993).

3. Analysis of Patulin in Apple Food for Children by DDMP. The samples (15 g of food) were mixed with 15 mL of distilled water and extracted using 60 mL of ethyl acetate placed in a 50-cm dialysis tube by shaking overnight at room temperature. The content of the dialysis tube was decanted and the aqueous layer removed. The volume of the organic extract was measured and dried. Five milliliters of the extract was evaporated to dryness, dissolved in 1 mL of chloroform, and treated in an ultrasonic bath during 8 min (Prieta, 1993).

SepPak Purification. The cleanup procedure was performed using the method of Rovira *et al.* (1993). The purified extracts were evaporated to dryness under a gentle stream of air. Apple juice extracts were dissolved in 1 mL of water–

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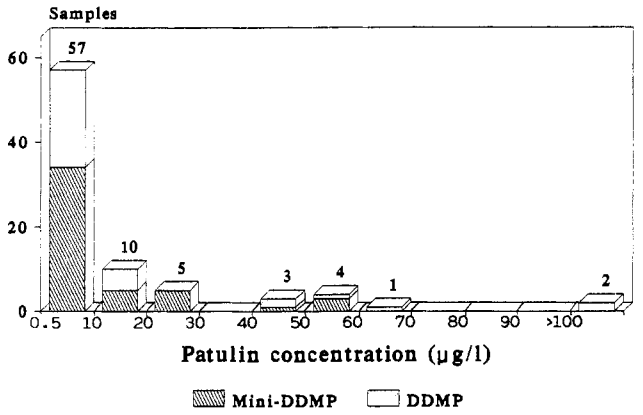

Figure 1. Histogram of the distribution of patulin values found in 82 positive samples of 100 analyzed commercial apple juices. Sixty samples were analyzed by mini-DDMP and 40 samples by DDMP. Recovery factors of 100/63 to mini-DDMP and 100/85 to DDMP were used to estimate the original patulin content.

THF (99+1) and children's apple foods extracts in 0.5 mL of water–THF (99+1).

HPLC Conditions. A mobile phase consisting of water–THF (99+1) was used at a flow rate of 1 mL/min. Detection was carried out at 275 nm.

RESULTS

Apple Juice. Sixty commercial apple juice samples were analyzed by mini-DDMP, and 40 samples were examined employing DDMP. To study the results as a whole, the original patulin content in apple juice was estimated by taking into account the average recovery at 20 μg/L of the employed methods. When the mini-DDMP was used, the average recovery at 20 μg/L was 63% and the minimum detectability was 0.5 μg/L in spiked samples (Prieta, 1993). Using DDMP, the minimum detectability was 1 μg/L and the average recovery at 20 μg/L was 85% in spiked samples (Prieta *et al.*, 1993).

Results obtained are shown in Figure 1. The incidence of patulin was high, being positive in 82% of samples; however, 69.5% of positive juices (57 samples) contained less than 10 μg/L and only 18.3% (15 juices) contained more than 20 μg/L. The patulin content ranged from 0.5 to 170 μg/L, the average amount being 13.8 μg/L.

Although Spain has no legislation on the subject, seven samples exceeded the limit recommended by the WHO (50 μg/L). Five of them contained between 50 and 61 μg/L, and two samples reached levels of 164 and 170 μg/L.

Apple Food for Children. When the DDMP method was used, the average recovery at 5 μg/kg was 81% and the minimum detectability was 1 μg/kg in spiked samples (Prieta, 1993). Patulin was not detected in any of the 12 samples of commercial children's apple food examined.

DISCUSSION

Various foodstuffs have been widely surveyed for the natural occurrence of patulin by many authors for the past 20 years. Although a variety of foods has been examined, only apple products, and especially apple juice, have been frequently found to be naturally contamined with patulin. Lower levels are generally obtained in other fruits and fruit products (Harrison, 1989; Kubacki, 1986).

The results obtained in this work are in accordance with those reported by other authors from other countries when analysis methods with minimum detectability of 1–2 μg/L for patulin in apple juice were employed. A survey conducted in Norway (Stray, 1978) showed that patulin was detected in 80% of 140 apple juice samples, ranging from 1 to 220 μg/L. A German survey (Wittkowski *et al.*, 1982) reported that 62% of 66 apple juice samples contained patulin levels ranging from 5 to 50 μg/L. Jelinek *et al.* (1989) reported two other surveys: (1) a German survey found patulin in 73% of apple juices ranging from 2 to 50 μg/L; (2) in a U.K. survey, 53% of apple juice samples were reported to have a patulin content ranging from 1 to 56 μg/L.

Recently, Prieta (1993) reviewed results of 20 surveys reported by several authors from 1974 to 1993. In all, 4298 apple juices were analyzed and 6–100% of samples (52% on average) contained detectable amounts of patulin ranging from 1 to 45 000 μg/L. In many of these surveys patulin determination was carried out using TLC; these methods have a detection sensitivity of 20–25 μg/L, lower than that obtained by HPLC. The highest concentration found for us was 170 μg/L; however, in 11 of 20 surveys reported by Prieta (1993) the maximum content exceeded this level.

Patulin was not found in fruit food for children. To the best of our knowledge, there have been two surveys of patulin performed in apple food for children: (1) Vesely *et al.* (1982) analyzed 40 samples, showing that one of them contained 50 μg/L of patulin; (2) Wittkowski *et al.* (1982) did not find patulin in any of 7 baby foods analyzed. In whole, of 47 examined samples, only 1 contained patulin.

In relation to the analytical techique employed in this work, the possibility to detect patulin levels of 0.5–1 μg/L in apple products, values 50–100 times lower than maximum value permitted for patulin by the health authorities of most of the countries having regulations (van Egmond, 1989), provides a wide safety margin for the determination of patulin in apple products by DDMP. For mini-DDMP the minimum detectability was 0.5 μg/L; to the best of our knowledge, this is the lowest detectability described in a method for the determination of patulin.

On the basis of works reported previously (Prieta, 1993; Jelinek *et al.*, 1989), apple products, and especially apple juices, have the highest potential patulin risk. Since patulin is resistant during the processing of apple juice and fruit food for children, it can be used as an effective quality indicator of the fruits used in the process. Because of this, we suggest, as Norway authorities have regulated (Stoloff *et al.*, 1991), that patulin should be used for quality control purposes of food and drink elaborated with apples, indicating the quality of raw materials employed, mainly the presence of rotted apples.

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