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Short communication

Patulin in homogenized fruit's and tomato products

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ABSTRACT

Patulin is a mycotoxin produced by microscopic fungi belonging to the *Penicillium* and *Aspergillus* genera. The natural occurrence of patulin in baby food products marketed in Italy is frequently detectable in moldy fruits and their derivatives. The EC Regulation 1881/06 has limited the presence of patulin in baby food to 10 μg/kg or 10 μg/L on the basis of a Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.4 μg/kg BW set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). A total of 80 homogenized baby foods were analyzed to evaluate the exposure of babies to patulin through the consumption of these products. The experimental tests have revealed significant differences between the products from organic production and those in traditional production in all the categories analyzed. The tomato concentrates showed an average of patulin concentration of 7.15 ng/ml of product; the tomato sauce of 4.05 ng/ml; the tomato sauce to the baby foods of 5.23 ng/ml; the homogenized apple of 0.85 ng/ml; the homogenized pear of 0.79 ng/ml. The tomato sauce conventional *vs* organic crops showed an average of 5.75 *vs* 3.49 ng/ml, respectively; the homogenized pear conventional *vs* organic ones of 0.72 *vs* 0.76 ng/ml, respectively; the homogenized apple conventional *vs* organic ones of 1.92 *vs* 0.13 ng/ml, respectively. The low incidence of the patulin level in Italian products is a clear parameter to establish the quality of the fruits and their derived products.

1. Introduction

Mycotoxins are secondary bioactive metabolites produced mainly by the mycelial structure of filamentous fungi, or more specifically, the molds. The biochemical significance of mycotoxins in fungal growth and development is not yet clear (Moss, 2002).

Patulin (4-hydroxy-4H-furo[3,2-c]pyran-2[6H]-one) is present globally in a wide range of fruit and vegetables.

It has been identified in different types of fruits (apples, pears, peaches, cherries, black currants, oranges, apricots, pineapple, grapes, bananas, strawberries, plums) (Buchanan et al., 1974; Frank, 1977; Karabulut & Baykal, 2002; Scott, Miles, Toft, & Dobe, 1972 and 1977), but was especially found in apples, pears, peaches, and in their processed products like juices and puree (World Health Organization, 1996). The relevance of Patulin among important mycotoxins cannot easily be underestimated, and it is synthesized by several species of filamentous fungi belonging to the genera *Penicillium*, *Aspergillus*, and *Byssochlamys* (Jimenez, Sanchis, Mateo, & Hernandez, 1988; Stray, 1978).

Penicillium expansum is considered the most important producer of patulin (Firsvad and Thrane, 1996), and it is commonly identified as the "blue mold rot" found in storage-rotted apples (Bompeis and Cholodowski-Faivre, 2000; Doores, 1983). *P. expansum* develops often on the surface of healthy fruit (Sydenham et al., 1995), but is normally associated with damaged fruits or fruits already infected by other microorganisms in orchards and postharvest conditions (Buchanan et al., 1974; Wilson and Nuovo, 1973).

The presence of patulin in these products is due to fungal contamination found in primary production, to little resistance of certain cultivars to pests attack or adverse weather conditions, but also to the storage conditions of raw materials (Burda, 1992; Ueno, 1987).

Several scientific evidences in animals, regarding the effects associated to the ingestion of patulin in the long term, have shown immunotoxicity (Escoula, More, & Baradat, 1977, 1988a, 1988b; Paucod, Krivobok, & Vidal, 1990), mutagenicity, neurotoxicity (Devaraj, Radha-Shanmugasundaram, & Shanmugasundaram, 1982), genotoxicity (Bürger, Brakhage, Creppy, Dirheimer, &

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Oschenthaler, 1988; Mori et al., 1984), and teratogenicity (Ciegler, Beckwith, & Jackson, 1976; Roll, Matthiaschk, & Korte, 1990). Although patulin is considered to be mutagenic, in 1993 it was included in Group 3 by the International Agency for Research on Cancer among those substances "not classifiable as a carcinogen to humans" (IARC, 1986). The EC Regulation 1881/2006, which indicates maximum levels for certain contaminants in food, sets the maximum limit of patulin equal to 10 μg/kg (or 10 μg/l) in apple juice and solid apple products, including apple compote and apple puree, for infants and young children and labeled and sold as such, and in baby foods other than processed cereal-based foods for infants and young children. The homogenized baby foods are considered to be food ready for use, sterilized and hermetically packaged, prepared from properly controlled nutrients, and subjected to a homogenization process to improve the ingestion and digestion.

In view of their wide use, both because they supply the nutritional needs for children, and because they consent easiness and rapidity in preparing meals, several samples of fruit-homogenized baby food were collected from Italian markets to analyze and assess the patulin presence and their compliance with the limits provided by the law.

2. Materials and methods

Patulin standard was purchased from Sigma Chemical Company (St. Louis, MO, USA). Water for the high-performance liquid chromatography (HPLC) mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA, USA). All other chemicals and solvents were HPLC grade from Sigma Chemical Company. A total of 80 homogenized fruit and vegetable-based baby food products were purchased in different Italian supermarkets.

From the stock solution consisting of 5 mg of patulin dissolved in 25 ml of ethylacetate, 1 ml was taken, transferred into a 10 ml flask, and the solvent was evaporated to dryness under a nitrogen stream. Acetonitrile was immediately added to dissolve the residue and to obtain a patulin work solution of 200 μg/L.

The work solution was stored at −20 °C and was evaluated by UV spectra taken by the Diode Array Detector and the wavelength set in the range from 230 to 320 nm.

To validate the method, an analytical curve was constructed with standard patulin solutions of different concentrations and in triplicates: 5, 10, 20, 27, 30, 40 and 50 ppb. Samples of baby food were fortified with the patulin solution at different concentrations used for the analytical curve construction (5, 10, 20, 30, 50 ppb), in triplicates, and the chromatographic analysis was performed in duplicate. The results in Table 1 show the percent recovery and their precision data expressed as the average of results obtained by the fortification of the 5 samples for each concentration of standard patulin added.

All samples including the standard patulin solutions were diluted in a mixture of acetonitrile/water (95:50, v/v) for the HPLC analysis.

Table 1
Recovery and precision data for extraction of patulin from baby food spiked at 5, 10, 20, 30 and 50 ppb.

Spiked concentration [PAT] (ppb)	Recovery (%)	SD (%)
5	96.03	0.32
10	97.47	0.80
20	95.60	0.66
30	96.43	0.71
50	95.40	1.67

Liquid chromatography analysis: an Agilent R® (Santa Clara, CA, USA) 1100 Series equipped with pumps, a Rheodyne Model 7125 injector (20 μL loop) and a DAD detector were used. A LC column Restek R® (Bellefonte, PA, USA) C18 (5 μm) (250 × 4.6 mm i.d.) was used with a mobile phase consisting of a mixture of water (A): acetonitrile (B), degassed at a flow rate of 1 mL/min. The following step elution was used: 0–19 min, 16% mobile phase B; 20–38 min, 50% mobile phase B; 43–50 min, 16% mobile phase B. The Diode Array Detector wavelength was set in the range of 230–320 nm. The analysis was performed at 37 °C. Under these conditions, patulin elutes at about 18–20 min.

Samples were processed in duplicate according to the procedure described by Iha and Sabino (2008), that was used as reference method. Briefly, 5 mL aliquots of homogenized juice samples were pipetted into 15 mL centrifuge tubes containing 0.5 g sodium bicarbonate and 5 mL ethyl acetate:hexane (96:4). The centrifuge tubes were closed and vigorously shaken for 5 min and, after centrifugation for 1 min at 580 g, 3 mL of the organic phase were transferred to tubes containing 30 ml acetic acid. The mixture was evaporated to dryness in a nitrogen stream and the residue immediately dissolved in 1 ml of 0.1% acetic acid (pH 3.2) in a Vortex mixer (Fanem) for 1 min.

The solution containing the extracted material was filtered through a 0.45 μm filter (Merck, Darmstadt, Germany) before being chromatographed in 50 μl aliquots.

All samples were filtered through a 0.22 μm syringe filter (Millipore) prior to injection (20 μL) onto the column. Mycotoxin quantification was carried out by comparing peak areas of investigated samples to the calibration curve of standards.

3. Results and discussion

In the literature, several methods are used to extract and analyze patulin from food sources such as juice, purees, or others. One of the most common extraction protocols requires ethyl acetate to extract patulin from juice and then washing the organic solution with sodium carbonate to remove potentially interfering phenolic compounds (Gokmen and Acar, 1996).

Other authors slightly modified this method introducing a solid phase extraction prepurification with silica before HPLC analysis (Rovira, Ribera, Sanchis, & Canela, 1993). These procedures are fast and inexpensive, however they may destroy patulin, whose lactone structure is not stable at basic working pH. In addition, these protocols have some problems with no clarified juice or complex matrixes such as baby food or mousse. MacDonald, Long, Gilbert, and Felgueiras (2000) suggest to use an enzymatic approach. Pectinase removes pectines from juice before HPLC analysis, and the digested solution is easier to analyze because a large part of interferences is eliminated from solution. This extraction and prepurification procedure requires at least 2 h at 40 °C or incubation overnight at room temperature.

This protocol has an economic cost for enzyme and requires a long time to obtain a partial clarification of sample juice. For these reasons, this method does not appear useful for large sampling and routine surveillance of apple products. Malone, Humphrey, Fleetwood, and Romer (1998) suggested pre-purifying the samples through Mycosep columns, that retained interfering impurities, and a good recovery (82–96%) was obtained. Several extraction methods were reported in the literature with good recoveries, using silica gel, florisil, Celite columns as prepurifying columns; a general limit of quantitation is considered 10 μg/mL (Sewram, Nair, Nieuwoudt, Leggott, & Shephard, 2000).

For more complete information, a table of the chemical-nutritional composition of the samples analyzed by category is provided below (Table 2).