Occurrence of Patulin in Apple Juice

The natural occurrence of patulin in a commercial sample of apple juice was demonstrated for the first time. The concentration was 1 ppm. Identification was by thin-layer and gas chromatography and mass spectrometry on successively more purified material.

Patulin {4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one} is a toxic antibiotic produced by several species of *Aspergillus* and *Penicillium* (Korzybski *et al.*, 1967). Dickens and Jones (1961) found that patulin caused tumors in rats when given by subcutaneous injection, and it has been shown to be mutagenic and to cause chromosome damage in other biological systems (Mayer and Legator, 1969). Thus the possibility that patulin could occur in foods made from fungal contaminated raw material has caused some concern. In particular, apples naturally rotted by *P. expansum* often contain patulin (Brian *et al.*, 1956; Harwig and Scott, 1971; Walker, 1969), leading to speculation that patulin could survive processing and be found in apple juice and other apple products. Patulin is relatively stable when added to apple juice and stored for up to 5 weeks (Scott and Somers, 1968; Pohland and Allen, 1970).

This communication describes the identification of patulin in a sample of bottled "sweet apple cider," which is actually unfermented apple juice, rather than a true cider. We had not detected patulin in 11 samples of commercial apple juice analyzed previously.

EXPERIMENTAL

Fifty milliliters of apple juice were extracted and analyzed by the method of Scott and Somers (1968). Thin-layer chromatography (tlc) was carried out on 20-cm × 20-cm × 0.3-mm layers of Adsorbosil 5 (Applied Science Laboratories, Inc.), which were developed in ether (equilibrated) or toluene–ethyl acetate–90% formic acid (30:15:6) and sprayed with 4% aqueous phenylhydrazine hydrochloride followed by heating at 110°C for 2–3 min. The amount of patulin estimated visually by comparing intensities of the yellow spots with standards at the same R_f (0.70 and 0.52, respectively, in the two solvent systems) corresponded to 1 ppm in the juice. Use of a second spray reagent, acidic ethanolic *p*-anisaldehyde (Scott *et al.*, 1970), revealed the presence of an interfering spot in the second solvent system, but indicated that ether would be suitable for isolation of patulin by preparative tlc. The patulin area, located by the phenylhydrazine spray at the side of the plate, was removed and eluted with redistilled methylene chloride–acetonitrile (1:1). Gas chromatographic analysis (*vide infra*) at this stage confirmed the tlc estimate of 1 ppm of patulin in the juice, although gas chromatography was useless for the initial extract because of interfering background material.

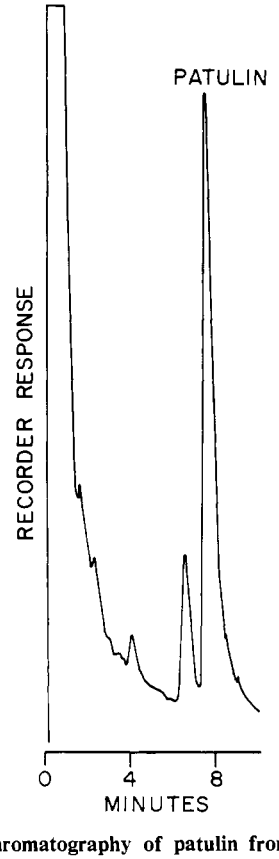

Figure 1. Gas chromatography of patulin from apple juice after tlc purification

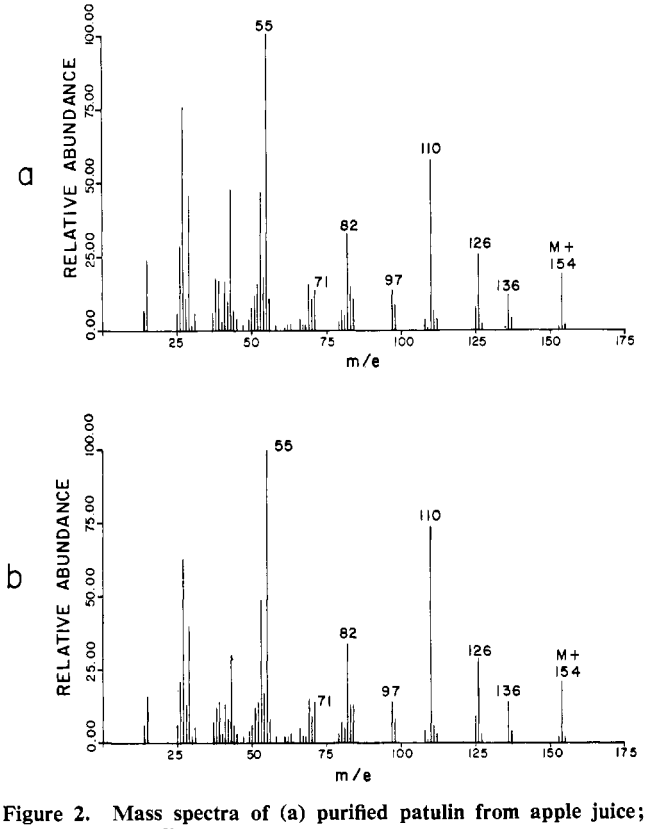

Figure 2. Mass spectra of (a) purified patulin from apple juice; (b) standard patulin

Approximately 20 μg of pure patulin were obtained by gas chromatography (Figure 1) of part of the tlc-pure material on a Hewlett-Packard F&M 810 instrument equipped with a flame detector and 9:1 stream splitter. Conditions were as follows. Column: 6-ft × 4-mm i.d. glass column packed with 5% OV 210 (Chromatographic Specialities Ltd., Brockville, Ontario) on 60–80 mesh Diatoport S (Hewlett-Packard). Injection port temperature: 250°C. Column temperature: 135°C. Detector temperature: 250°C. Helium carrier gas flow: 60 ml/min at 45 psi pressure. Patulin had a retention time of 7.6 min and was collected in a glass capillary (1 mm i.d.).

Mass spectra (Figure 2) of isolated patulin and standard patulin were obtained using the direct probe of a Hitachi Perkin-Elmer RMS-4 mass spectrometer, operating at 80 eV with an ion source temperature of 195°C and probe temperature of 120°C. The spectra were virtually identical after small corrections for machine background.

DISCUSSION

This is the first reported occurrence of patulin in a commercial food product. To evaluate patulin as a health hazard requires additional toxicological data (chronic oral feeding studies in animals are particularly lacking) and a year-round survey of apple-derived foods for this mycotoxin.

Gas chromatography of patulin has not previously been described, although the trimethylsilyl ether, acetate, and chloroacetate derivatives were used by Pohland *et al.* (1970). Assignment of structures to seven principal fragment ions in the mass spectrum of deuterated patulin was made by Scott and Yalpani (1967); an actual spectrum was not published.

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Identification of Abscisic Acid in Bartlett Pears and Its Relationship to Premature Ripening

A growth inhibitor in Bartlett pear was identified as abscisic acid (ABA) by paper chromatography, ultraviolet spectrometry, and gas–liquid chromatography. Fruit from limb enclosures kept at 16–24°C showed only a slight increase in the amount of ABA. Premature ripening and greater accumulation of ABA was found in fruit from branches kept at 7–18°C during 30-day preharvest period.

Exposure of Bartlett pears to abnormally cool temperature for short periods prior to harvest causes an early development and acceleration of the biochemical and physiological changes normally associated with maturation and ripening (Wang *et al.*, 1971). As a result, ripening is initiated and develops on the tree prior to anticipated time of normal harvest. While the nature of this physiological disorder is not fully understood, the level or ratio of certain growth substances within the fruit may be involved in stimulation of ethylene production and development of ripening capacity (Dilley, 1969).

Abscisic acid (ABA), known to stimulate fruit ripening (Addicott and Lyon, 1969), occurs in unripe Clapp's Favourite pears and increases in concentration during ripening (Rudnicki *et al.*, 1968). The present study was initiated to determine if ABA also occurs in the Bartlett pear cultivar and if significant changes in concentration develop during cold-induced premature ripening.

EXPERIMENTAL

The experiments were initiated 30 days prior to the estimated harvest date. Installation of Mylar covered limb enclosures and methods of temperature control were described previously (Wang *et al.*, 1971). Cooled cages were maintained at 18°C during the day and at 7°C during the night. Temperatures in the heated cages were maintained at 24°C daytime and 16°C at night. The first samples were collected 10 days after start of the experiment.

Extraction and purification of the sample followed the procedures described by Strausz (1970). The acidic ether fraction was found to contain the most inhibitory activity and was used exclusively in this study. The residue of the acidic ether fraction was separated by paper chromatography, using Whatman No. 1 and 2-propanol:ammonia:water (8:1:1 v/v/v). Bioassay procedures described by Nitsch and Nitsch (1956) were used. The activity of the growth inhibitor was determined by the coleoptile straight growth test using