

## *A Research Note*

# Patulin in Apple Juice from Roadside Stands in Wisconsin

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(Received for publication February 22, 1979)

### ABSTRACT

High-performance liquid chromatography was used to determine the amount of patulin in 40 samples of apple juice sold at roadside stands in Wisconsin. Samples were taken during juice-producing seasons in 1976 and 1977. Nine of 11 samples obtained in 1976 contained from a trace (approximately 10 µg) to 350 µg of patulin per liter. Fourteen of 29 samples obtained in 1977 contained from a trace to 204 µg of patulin per liter. The average concentration of patulin in all samples was 51 µg/l; most samples contained less than 50 µg/l.

Patulin [4-hydroxy-4H-furo (3,2c) pyran-2(6H)-one] is a toxic secondary metabolite produced by some molds in several genera of fungi. It is acutely toxic to mice (2) and is mutagenic to yeast cells (8). Patulin also acts as a teratogen (3) and produces tumors in rats (4).

Apples are sometimes rotted by the patulin-producing *Penicillium expansum* and hence occasionally contain patulin (1,6,10). Thus it is not unusual that investigators also found patulin in apple juice (9). Some researchers have done limited surveys on the frequency of occurrence and amount of patulin in apple juice. Ware et al. (10) noted that 8 of 13 commercial apple juices contained from 44 to 309 µg of patulin per liter. Patulin at concentrations of 6 to 16,400 µg/l was also found in 8 of 20 samples of home-made apple juice (6).

Work described in this report was done to determine if patulin occurred in apple juice as sold at roadside stands in Wisconsin.

### MATERIALS AND METHODS

Samples of apple juice were obtained from roadside stands or directly from producers. All juices were unpasteurized, unclarified and none contained additives.

Apple juice was tested for patulin using high-performance liquid chromatography, as described by Ware et al. (10). All samples were

kept refrigerated for no more than 2 days after collection and before analysis.

Chromatography was accomplished using a Waters pump (Model M6000A, Waters Assoc., Milford, MA), a Schoeffel detector (Model SF 770, Schoeffel Inst. Corp., Westwood, NJ) and a Whatman Partisil-10 column (Whatman Inc., Clifton, NJ). All solvents used in the analysis were obtained from Burdick and Jackson Laboratories (Muskegon, MI) and were of spectrophotometric grade. Solvents (Aldrich Chemical Co., Milwaukee, WI) used for extractions and column chromatography were analytical reagent grade.

### RESULTS

Results obtained from our tests are given in Table 1. Samples from 1976 contained an average of 115 µg of patulin per liter. This compares to 26 µg/l in samples from 1977. The amounts of patulin found in 1976 were more uniformly distributed over the entire range of concentrations than were the amounts found in samples from 1977. Most samples from 1977 contained less than 50 µg of patulin per liter. The difference in results from the 2 years is also illustrated by the smaller percentage of samples containing patulin in 1977 (48%) as compared to 1976 (82%). The ranges of values for samples containing patulin were approximately 10 to 350 µg/l in 1976 and 10 to 204 µg/l in 1977.

### DISCUSSION

Fifty eight percent of the samples tested in this study contained patulin. This was similar to the value of 61% obtained by Ware et al. (10) when they examined 13 samples. However, our results differed in that most of our samples had less than 50 µg/l, whereas the positive samples of Ware et al. had a minimum concentration of 44 µg of patulin per liter.

TABLE 1. Amounts of patulin in apple cider sold at roadside stands in Wisconsin (1976-77).

Concentration (µg/l)	1976		1977		Combined	
	No.	%	No.	%	No.	%
< 10	2	18	15	52	17	43
10	2	18	2	7	4	10
11-50	0	0	8	28	8	20
51-100	2	18	2	7	4	10
101-200	2	18	1	3	3	8
201-300	2	18	1	3	3	8
> 300	1	9	0	0	1	3
Ave. (µg/l)		115.2		26.0		50.7
Std. dev.		121.5		45.3		83.2

Differences noted for samples from 1976 and 1977 could have resulted from one or several factors. First, there may have been variability in sampling of juice. In 1977, a larger number of samples were obtained from more sources throughout the state than in 1976; samples in 1976 were obtained primarily from southern Wisconsin. Second, differences in environmental conditions (samples from northern and southern versus samples from only southern Wisconsin) to which apples and/or juice were exposed could account for some of the variation in results. Third, there may have been differences in quality control among producers and between years. It is possible that such factors as market prices or crop yields could have encouraged use of apples of lower quality in 1976 than in 1977. Fourth, there may have been differences in elapsed time between pressing of juice and taking of samples.

A question which has not been answered is that of health hazards resulting from patulin in apple juice. If one assumes that humans have the same oral LD<sub>50</sub> as do mice, 35 mg per kg of body weight, (2), 70-kg persons would each have to consume about 2.45 g of patulin to cause death of 50% of the persons in a given group. To get this amount of patulin it would be necessary to consume about 150 liters of juice which contains 16,400 mg of patulin (6) per liter. Thus the possibility of fatality from drinking apple juice contaminated with patulin is virtually nonexistent for healthy humans. However, one should keep in mind the possibility of effects other than death being caused by sublethal doses of patulin.

Although observations on humans are lacking for this aspect of patulin's toxicity, the fact that patulin is a possible carcinogen or teratogen should be enough reason to limit its content in foods.

There is evidence of cumulative toxic action when 25 mg of patulin per kg of body weight are given orally to mice daily for 14 days. This toxic action includes pulmonary edema in surviving mice (2). Other sublethal effects which have been noted in animals are accumulation of liquid in the peritoneal cavity and extensive hemorrhages (7).

Our results show that apple juice from roadside stands in Wisconsin contained patulin about as often as did commercial apple juice in the Washington D.C. area. Results also show that there was a wide range in amount of patulin in samples but most contained relatively small amounts of the mycotoxin.

#### ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by contributions from the food industry to the Food Research Institute. We thank Dr. M. N. Dana, Department of Horticulture, University of Wisconsin-Madison; the Wisconsin Department of Agriculture, Trade and Consumer Protection and numerous county extension persons for help in obtaining samples of apple juice.

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