Volatility of Patulin in Apple Juice

Robert A. Kryger*

Danisco Cultor USA, Inc., 4330 Drane Field Road, Lakeland, Florida 33811

Patulin is a mycotoxin produced by certain fungi, such as those found commonly on apples. The patulin content of apple juice is a regulatory concern because patulin is a suspected carcinogen and mutagen. A simple model of the apple juice concentration process was carried out to examine the possible contamination of patulin in apple aroma, a distillate produced commercially in the concentration of apple juice. The results show no evidence for patulin volatility, and document a reduction in patulin content by at least a factor of 250 in the apple distillate obtained from apple juice. Furthermore, a survey of several commercial apple aroma samples found no evidence of patulin content.

Keywords: *Apple (Pyrus malus); apple aroma; patulin volatility*

INTRODUCTION

Patulin (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one, $C_7H_6O_4$) is a chemical generated during the growth of certain fungi (especially of the genus *Penicillium*) on harvested apples (*Pyrus malus*) (*1*). The structure of patulin is shown in Figure 1. When patulin contaminated apples are processed for juice, the patulin may be introduced into the juice, and subsequently into the human food chain. In recent years, the patulin content of apple products has been of particular interest because research has shown that patulin is a possible carcinogen and mutagen (*2*, *3*). Consequently, government food safety organizations worldwide are proposing limits on patulin content in apple juice products (*4*, *5*). The proposed regulations typically limit patulin content in apple juice to not more than 50 μg/kg (ppb). Concentrated apple juice is evaluated after dilution back to single-strength juice level.

Apple aroma is another product produced during the processing of apples into apple juice concentrate. After the apples are pressed and the juice is removed from the pomace, the juice is heated under vacuum to remove the water and form the juice concentrate. The water removed from this stripping is further fractionated in a continuous process to concentrate the most volatile flavor components. The volatile fraction, typically concentrated a factor of 150 times compared to the starting juice, is collected and sold as apple aroma. Commercial apple aroma is mostly water with a few percent ethanol and part-per-million (ppm) levels of various aroma compounds. Most aroma is added back to apple juice to restore the natural flavor. Apple aroma is also used as a natural flavoring ingredient for other food applications.

On the basis of a reasoned analysis of the aroma collection process, one would expect that patulin would not be present in apple aroma. Patulin has a MW of 154 and is less volatile than water. Apple aroma represents only the most volatile fraction separated from the apple juice and is highly concentrated compared to the starting juice. Consequently, patulin should not be preferentially stripped from the juice, and even if it were volatilized in small quantities, it should not be fractionated into the aroma. Unfortunately, no published data on the patulin content of apple aroma could be found. Furthermore, Lindroth and Niskanen reported high levels of patulin measured in unspecified "apple flavor" samples imported in Finland (*6*), although it is not clear whether these samples contained apple aroma. Because of the human health concerns, an experimental verification of the mechanics of patulin distillation as applied to apple aroma collection was warranted.

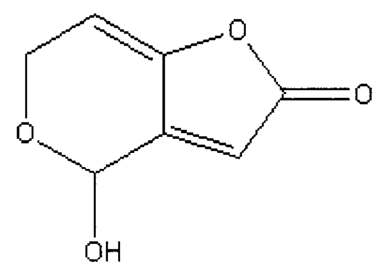

Figure 1. The structure of patulin.

Consequently, the current goal was to model the collection of apple aroma during the concentration of apple juice containing a known level of patulin. Analysis of the patulin content of the various fractions would provide experimental evidence to support the hypothesis that patulin content in apple aroma is insignificant. As a further test, a set of commercial apple aroma samples representing production in various apple-growing regions worldwide were analyzed for patulin content.

MATERIALS AND METHODS

Apple juice concentrate with a relatively high level of patulin was purchased from a commercial source. The concentrate was reported by the supplier to have a patulin level of 90 ppb (when diluted to 11.5° Brix) and to be of South American origin. The concentrate was 70.2° Brix. For the purpose of generating single-strength apple juice for the present study, the concentrate was diluted to 11.5° Brix using deionized water. To a 3-L sample of this juice, 10 mg of patulin (Sigma) was added to bring the patulin level to approximately 3420 ppb. After addition of the solid patulin, the juice was stirred for 1 h to

* To whom correspondence should be addressed (telephone 863-646-0165; fax 863-646-0991; e-mail ggrak@danisco.com).

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Table 1. Patulin Content of Experimental Samples

sample	description	patulin (ppb)
	Patulin Recovery Experiment	
A	starting spiked single-strength apple juice (unprocessed)	3240
B	juice after heat treatment	2180
	Spiked-Juice Distillation Experiment	
C	apple distillate cut 1	< 10
D	apple distillate cut 2	< 10
E	reconstituted juice after distillation	2500
	Unspiked-Juice Distillation Experiment	
F	starting unspiked single-strength apple juice (unprocessed)	84
G	apple distillate cut 1	< 10
H	apple distillate cut 2	< 10
I	reconstituted juice after distillation	69
	Apple Aroma Samples	
	apple aroma ex China (supplier 1)	< 10
	apple aroma ex China (supplier 2)	< 10
	apple aroma ex Chile	< 10
	apple aroma ex Argentina	< 10

ensure uniform mixing and then stored under refrigeration (4 °C) until used. A sample of this spiked juice (A) was analyzed for patulin content (as described below).

To study the stability of patulin in the apple juice matrix under the present experimental conditions, a 1-L glass flask was filled with 500 g of spiked apple juice. This flask was sealed under an air headspace with a ground-glass stopper. The stopper joint was tightly wrapped with Teflon tape to ensure an airtight seal. The sealed flask was then heated for 177 min in a hot-water bath (100 °C) under conditions similar to those used for the distillation experiment below. After the sealed flask was heated, it was cooled and stored under refrigeration prior to being opened for patulin analysis (sample B).

The apple aroma distillation process was modeled using a standard laboratory rotary evaporator (Büchi model W240). The single-strength juice was poured into the evaporator pot (approximately 1000 g) and distilled using a hot-water bath (100 °C) as a heat source. The distillate was condensed using chilled glycol coolant (about 4 °C). The distillation process (with fixed bath temperature) was started at atmospheric pressure. The pressure was slowly reduced using a vacuum pump to maintain approximately constant boiling as the water was removed from the juice and the Brix level of the pot increased. Two separate distillations were run as described below; in each case the distillation lasted between 2.5 and 3 h and the pressure ranged from atmospheric to 0.35 bar.

In the first experiment, spiked juice (1008 g) was distilled and two cuts of approximately equal mass were collected: the first receiver collected 390 g of distillate and the second collected 422 g. During the first cut, the evaporator pressure ranged from atmospheric to 0.5 bar, and the second cut was taken between 0.5 and 0.35 bar. The two distillate cuts (samples C and D) were stored under refrigeration until analyzed. A third sample (E) was prepared by reconstituting single-strength juice using the concentrated juice pot and the two distillate fractions. Each of these samples was analyzed for patulin content.

For the second distillation experiment, single-strength apple juice was prepared as described above without the additional spiked patulin. This sample (F) was used as the starting material for concentration (1007 g). During the distillation process, 400 g of distillate was collected in the first fraction (sample G), and 436 g was collected in the second fraction (sample H). A final sample (I) was reconstituted from the final juice pot (67.6° Brix) by the addition of deionized water to bring the mixture back to single-strength juice (11.5° Brix).

Finally, four samples of commercial apple aroma were selected and tested for patulin content. The samples were randomly selected from commercial stocks representing the two largest apple-growing regions. Two samples were selected from Chinese apple producers and two were from South American producers. All four samples were representative of typical commercial apple aroma as determined by standard analytical tests (ethanol content, volatile components present, and flavor).

Patulin determination in the apple juice, distillate, and aroma samples was carried out according to the AOAC method 995.10 (*7*). Samples were extracted using ethyl acetate to remove the patulin. The ethyl acetate phase was dried using sodium sulfate and evaporated to dryness. To the dried extract, 0.5 mL of water (adjusted to pH 4.0 with acetic acid) was added. Patulin levels were determined by HPLC analysis of the solubilized extract using UV detection at 276 nm. Details of the extraction procedure and HPLC method are given in ref 7. External calibration standards were prepared and run at the same time as the experimental samples. The method as implemented had a lower limit of detection of 10 ppb patulin and an estimated uncertainty of quantitation of ± 10%. Uncertainties were estimated by running selected samples multiple times at the beginning, middle, and end of the runs. The standard deviation of the patulin levels for these standards ranged between 2 and 10%. Three of the samples with undetected levels of patulin were reanalyzed after spiking with known levels of patulin. The measured recovery of patulin ranged between 97 and 100% for these samples.

RESULTS AND DISCUSSION

The unprocessed spiked apple juice (sample A in Table 1) was found to contain patulin at 3240 ppb, consistent with the expected level of 3420 ppb. In contrast, sample B obtained from the heated, sealed flask showed a level of only 2180 ppb. Because no vapors could escape from the sealed flask, the results show that patulin degrades or reacts when heated to 100 °C for extended periods of time in apple juice. After 177 min of heating, only 67% of the patulin remained. Other authors have also reported evidence of patulin loss in apple juice under various conditions of heat treatment (*1, 8*−*9*).

The patulin content of each of the samples from the first distillation experiment are reported in Table 1. The unprocessed starting material (sample A) consisted of apple juice spiked with patulin to 3240 ppb, more than 60 times the proposed 50 ppb limit for apple juice (*4*) and well above typical values found in commercial apple juice. Because the minimum level of detection of the patulin level was relatively high (10 ppb), a high starting patulin level was chosen to increase the sensitivity of the experiment to patulin carry-over in the distillate.

The juice sample E, reconstituted from both distillation fractions of the first experiment, provides a simple test of the total patulin recovery. In the absence of any patulin loss, this sample should be identical to the starting juice. A patulin level of 2500 ppb was measured, accounting for 77% of the starting patulin. This value is consistent with the recovery yield found in the sealed system described above and suggests that the distillation process introduced no additional patulin-loss mechanisms.

No evidence for patulin content was observed in either distillate sample C or D. Two cuts were taken to look for evidence of patulin fractionation during the distillation process. However, with the absence of any measurable patulin in either distillate, one can conclude that the patulin content of the distillate is reduced by at least a factor of 250 compared to the starting juice.

The second distillation experiment was carried out using unspiked juice, which contained a patulin level closer to typical levels. The patulin levels of the samples obtained in this experiment are shown in Table 1. Here, the starting juice (sample F) contained 84 ppb patulin, in good agreement with the purported level of 90 ppb indicated by the commercial supplier. The reconstituted juice after distillation (sample I) was prepared using only the final pot and deionized water. The distillate cuts were not added back. In this way, the residual patulin was measured in the pot without the influence of possibly low levels of patulin in the distillate. The resulting patulin recovery yield of 82% (69 ppb out of 84 ppb) agrees well with the residual yields seen in the other experiments described above. The distillate cuts themselves again show no evidence of patulin content, although in this case the limit of detection is only 8 times less than the starting patulin level. No evidence was found that the patulin behaves differently in the unspiked juice than it does in the spiked juice.

As an additional check of the results, four commercial apple aroma samples were analyzed for patulin content. These samples were randomly chosen, typical, apple aroma lots from the largest apple growing regions and consequently should be representative of most commercial aromas. The results are seen at the bottom of Table 1. Although there was no way to ascertain the patulin level of the apple juice processed to produce this aroma, a check of random samples provides an additional estimation of the likelihood of finding patulin-contaminated aroma. In the present case, no detected patulin was found in any of the aroma samples.

The implication of these results for the safe use of apple aroma can be summarized here. The current experiments document that a reduction factor of at least 250 occurs in patulin level in apple distillate compared to that of the starting juice. The commercial production of apple aroma involves a more complex fractionation of the distillate to separate the water from the most volatile flavor fraction than that employed in the present experimental conditions. This fractionation step should further suppress patulin carry-over into the apple aroma. As apple aroma is typically used at a level of 0.7% in a single-strength juice, the aroma would need to contain over 7000 ppb patulin to deliver 50 ppb of patulin to the final beverage. On the basis of these results, the apple juice producing this aroma would require over 10^6 ppb patulin − many times over the highest levels reported in survey studies of apple juice patulin content (*1*). Thus, it seems safe to conclude that in all except the most unusual production circumstances, apple aroma should be effectively free of patulin contamination.

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LITERATURE CITED

(1) Harrison, Mark A. Presence and Stability of Patulin in Apple Products: A Review. *J. Food Safety* **1989**, *9*, 147−153.

(2) IARC. Patulin. *IARC Monogr. Eval. Carcinog. Risk Chem. Humans***1986**, *40*, 8398.

(3) WHO. Patulin. *WHO Food Addit. Ser.* **1990**, *26*, 143−165.

(4) FDA Draft Policy Guide: Apple Juice, Apple Juice Concentrates, and Apple Juice Products − Adulteration with Patulin, and the related background paper Patulin in Apple Juice, Apple Juice Concentrates and Apple Juice Products. *Fed. Regist.* **2000**, *65*, (117), 37791−37792. Also available at http://vm.cfsan.fda.gov/.

(5) Codex Alimentarius Commission of the FAO/WHO. Proposed Draft Codex General for Fruit Juices and Nectars, Document CL 2000/01FJ. 2000.

(6) Lindroth, S.; Niskanen, Ao. Comparison of Potential Patulin Hazard in Home-Made and Commercial Apple Products. *J. Food Sci.* **1978**, *43*, 446−448.

(7) AOAC Official Method 995.10 Patulin in Apple Juice. *J. AOAC* **1996**, *79*, 451.

(8) Kubacki, S. J. The Analysis and Occurrence of Patulin in Apple Juice. In *Mycotoxins and Phycotoxins*; Steyn, P. S., Vleggaar, R., Eds.; Elsevier Science: Amsterdam, The Netherlands, 1986; pp 293−304.

(9) Scott, P. M.; Somers, E. Stability of Patulin and Penicillic Acid in Fruit Juices and Flour. *J. Agric. Food Chem*. **1968**, *16*, 483−485.

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