Apple Quality, Storage, and Washing Treatments Affect Patulin Levels in Apple Cider

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ABSTRACT

Patulin is a mycotoxin produced primarily by *Penicillium expansum,* a mold responsible for rot in apples and other fruits. The growth of this fungus and the production of patulin are common in fruit that has been damaged. However, patulin can be detected in visibly sound fruit. The purpose of this project was to determine how apple quality, storage, and washing treatments affect patulin levels in apple cider. Patulin was not detected in cider pressed from fresh tree-picked apples (seven cultivars) but was found at levels of 40.2 to 374 µg/liter in cider pressed from four cultivars of fresh ground-harvested (dropped) apples. Patulin was not detected in cider pressed from culled tree-picked apples stored for 4 to 6 weeks at 0 to 2°C but was found at levels of 0.97 to 64.0 µg/liter in cider pressed from unculled fruit stored under the same conditions. Cider from controlled-atmosphere-stored apples that were culled before pressing contained 0 to 15.1 µg of patulin per liter, while cider made from unculled fruit contained 59.9 to 120.5 µg of patulin per liter. The washing of ground-harvested apples before pressing reduced patulin levels in cider by 10 to 100%, depending on the initial patulin levels and the type of wash solution used. These results indicate that patulin is a good indicator of the quality of the apples used to manufacture cider. The avoidance of ground-harvested apples and the careful culling of apples before pressing are good methods for reducing patulin levels in cider.

Patulin (4-hydroxy-4*H*-furo[3,2c]pyran-2[6*H*]one) is a mycotoxin produced primarily by *Penicillium expansum,* a mold responsible for blue mold rot in apples, pears, and other fruits. *P. expansum* is a ubiquitous fungal contaminant that can be isolated from the surface of healthy fruit. Normally, the growth of the fungus and the production of patulin are associated with fruit that has been damaged in a number of ways including through insect damage, storm damage, and handling procedures *(35)*. Although patulin can be present in visibly sound apples intended for direct consumption, it is typically found at higher levels in less-than-sound fruit destined for processing into juice, cider, sauces, purees, and jellies *(15)*.

Numerous surveys on the incidence and concentration of patulin in processed apple products have been published *(9, 13, 19, 27, 29, 45, 49)*. In surveys of apple products obtained in Turkey and New South Wales, Yurdun et al. *(49)* and Burda *(9)* reported that >25% of juice samples contained over 50 µg of patulin per liter, and several samples contained 500 to 1,000 µg of patulin per liter. In a survey of apple juices purchased between 1994 and 2000 in the United States, it was found that 12.6% of these juices had patulin levels of >50 µg/liter and that approximately 6% had levels of >100 µg/liter *(29)*.

Patulin is mutagenic *(23),* exhibits adverse effects on developing fetuses in rats *(12),* is diabetogenic to rats *(14),* and causes immunotoxic *(16, 31)* and gastrointestinal effects in rodents *(24).* Based on the results of reproductive toxicity studies and long-term toxicity studies involving animals, the Joint Food and Agriculture Organization–World Health Organization Expert Committee on Food Additives established a provisional maximum daily intake of 0.4 µg/kg of body weight for patulin *(46).* Because of potential adverse effects arising from long-term exposure to patulin, several trade organizations, such as the International Fruit Juice Association and the National Food Processors Association, recommend that limits be placed on patulin levels in apple products *(35).* At least 10 countries have established action levels of 50 µg/liter for patulin in apple juice, and several have established lower limits (25 to 35 µg/liter) *(42).* The U.S. Food and Drug Administration (FDA) has recently established a 50-µg/liter action level for patulin in single-strength and reconstituted apple juice *(40).*

Several methods for reducing patulin levels in juice products have been studied. The removal of decayed or damaged fruit or the trimming of moldy portions of apples prior to processing can reduce patulin levels in apple juice *(5, 21, 35–37).* Postpressing treatments, such as the addition of ascorbic acid *(7)* to cider or the filtration of cider through beds of charcoal and polymers *(1, 17, 35),* result in small to modest (10 to 60%) reductions in patulin levels. Despite these studies, little is known about the effects of apple quality and harvesting method on patulin levels in cider. The

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objectives of this study were (i) to determine how the apple harvesting method and culling affect patulin levels in cider pressed from fresh (nonstored) apples, (ii) to determine how culling affects the patulin content of cider made from stored apples, and (iii) to determine whether apple-washing treatments can reduce patulin levels in cider.

MATERIALS AND METHODS

Chemicals. Patulin and 5-hydroxymethyl furfural (HMF) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Other lab chemicals (analytical grade or high-performance liquid chromatography [HPLC] grade) were purchased from Fisher Scientific (Chicago, Ill.).

Cider production. Seven different apple cultivars (MacIntosh, Red Delicious, Golden Delicious, Gala, Fuji, Red Rome, and Granny Smith) were harvested from orchards in northern California. Apples were classified according to method of harvest (tree picked or ground harvested). Tree-picked apples were divided into two treatment groups: culled and not culled. Visibly damaged apples (those showing mold, major bruising, skin breaks, hail damage, or bird pecks) were removed from the culled group. In the unculled group, 10 to 70% of the apples were visibly damaged. The extent of apple defects varied with apple variety and time of harvest.

Three 91-kg (200-lb) batches of apples representing each variety and method of harvest were processed into cider at a cider mill in Placerville, Calif., that was operated by the El Dorado County Department of Agriculture. Apples were crushed in a hammer mill, and the resulting pulp was pumped to a cider press. Cider was obtained by pressing the pulp through a set of porous press cloths. The cider was then collected and pumped into a bottling tank. Three 50-g samples from each batch of cider were frozen until they were analyzed for patulin content.

Storage study. Tree-picked apples of several cultivars were put in cold storage (0 to 2°C) for 4 to 6 weeks. Controlled atmosphere (CA)–stored Fuji apples (storage conditions not known) were obtained from a commercial source. After storage, apples were divided into two groups, culled and not culled. Batches that were not culled contained apples with visible damage and mold. Ten to 70% of apples in unculled batches were visibly damaged as described above. Batches of apples (91 kg) were pressed into cider as described above.

Washing study. Batches (91 kg) of ground-harvested Golden Delicious apples (not culled) containing different initial patulin levels received one of three treatments before pressing: (i) no wash, (ii) a chlorine (100 or 200 ppm) solution wash, or (iii) a potable-water wash. Apples were washed in a dump tank containing 500 gal (1,900 liters) of cleaning solution. Apples were immersed in the wash solutions for ca. 2 min with no agitation. The wash solutions were drained from the apples, and the apples were then pressed into cider as described above. Duplicate trials were carried out for most wash treatments.

Patulin analysis. Cider samples were frozen and sent to the National Center for Food Safety and Technology (Summit-Argo, Ill.), where they remained frozen until they were analyzed for patulin. Patulin levels in cider samples were measured by the method of Brause et al. *(8).* A Waters (Milford, Mass.) HPLC equipped with a Model 600 pump, a Model 996 photodiode array detector (scanning at wavelengths of 210 to 400 nm), and Millenium 2010 software was used to identify and quantify patulin in cider samples. Separations were carried out with a Waters Resolve

C18 column (5 μm; 3.9 mm by 300 mm) and precolumn. The mobile phase consisted of 10% acetonitrile in distilled, deionized water, and the flow rate was 0.5 ml/min. This mobile-phase composition was chosen to prevent the coelution of patulin and (HMF) peaks. All cider samples were spiked with 50 μg of patulin per liter for the recovery studies. Patulin levels in cider samples were corrected for recovery on the basis of recovery data for each cider sample.

Two methods were used to verify the presence of patulin in cider samples. First, UV spectra of suspected peaks obtained during HPLC analysis were compared with peaks for patulin standards. Second, liquid chromatography–mass spectrometry (LC-MS) was used to verify the presence of patulin. A Waters HPLC pump (Alliance 2690 Separation Module) was used to supply a 0.5-ml/min flow of solvent (10% acetonitrile in water) through a Waters Resolve C18 column/precolumn. The entire 0.5-ml/min effluent from the column was directed into the electrospray ion source of a Micromass (Waters) Platform LC. The ion source was operated with a cone voltage of 25 V, a probe voltage of 3.5 kV, an N_2 gas pressure of 80 lb/in^2, and a source temperature of 120°C. The instrument was operated in the negative ion mode and scanned over a mass/charge ratio range of 25 to 200. Data were analyzed with Microlynx 3.4 software (Waters). Peaks were identified by comparing retention times and mass/charge ratios with those of patulin standards.

Statistical analysis. All patulin analyses were conducted in triplicate. Minitab statistical software was used to calculate means and standard deviations. Differences in means were determined with one-way analysis of variance followed by Tukey's multiple-comparison test. Spearman's rank correlation coefficients were calculated with the SAS statistical program (SAS Institute, Cary, N.C.).

RESULTS AND DISCUSSION

Patulin analysis. HPLC analysis of patulin in cider by the method of Brause et al. *(8)* resulted in chromatograms with baseline resolved peaks for HMF (retention time = 10.5 min) and patulin (retention time = 12.8 min) (Fig. 1). The mobile phase and the column used in the HPLC procedure were selected to yield maximum separation from interfering compounds, especially HMF, in cider. Since the cider samples were not heat processed (e.g., pasteurized), HMF, a nonenzymatic browning product, was present at low levels in most samples. Patulin with which cider samples had been spiked (at 50 μg/liter) was recovered at a level of 82.9 ± 7.4%. The limit of detection for patulin in the cider samples was 1 to 3 μg/liter.

The identity of each suspected patulin peak was confirmed by comparing the UV spectrum of the peak with that of the patulin standard. Figure 1 indicates that the UV spectrum for the suspected patulin peak in one cider sample (chromatogram C) was identical to the spectrum for the patulin standard (chromatogram B). LC-MS was used to further confirm the presence of patulin in cider samples. The retention times and mass/charge ratios of the peaks indicated the presence of patulin (retention time = 11.3 min; mass/charge = 153) in samples presumed to contain the toxin.

Patulin levels in cider pressed from fresh apples. The effects of harvesting method and culling on patulin
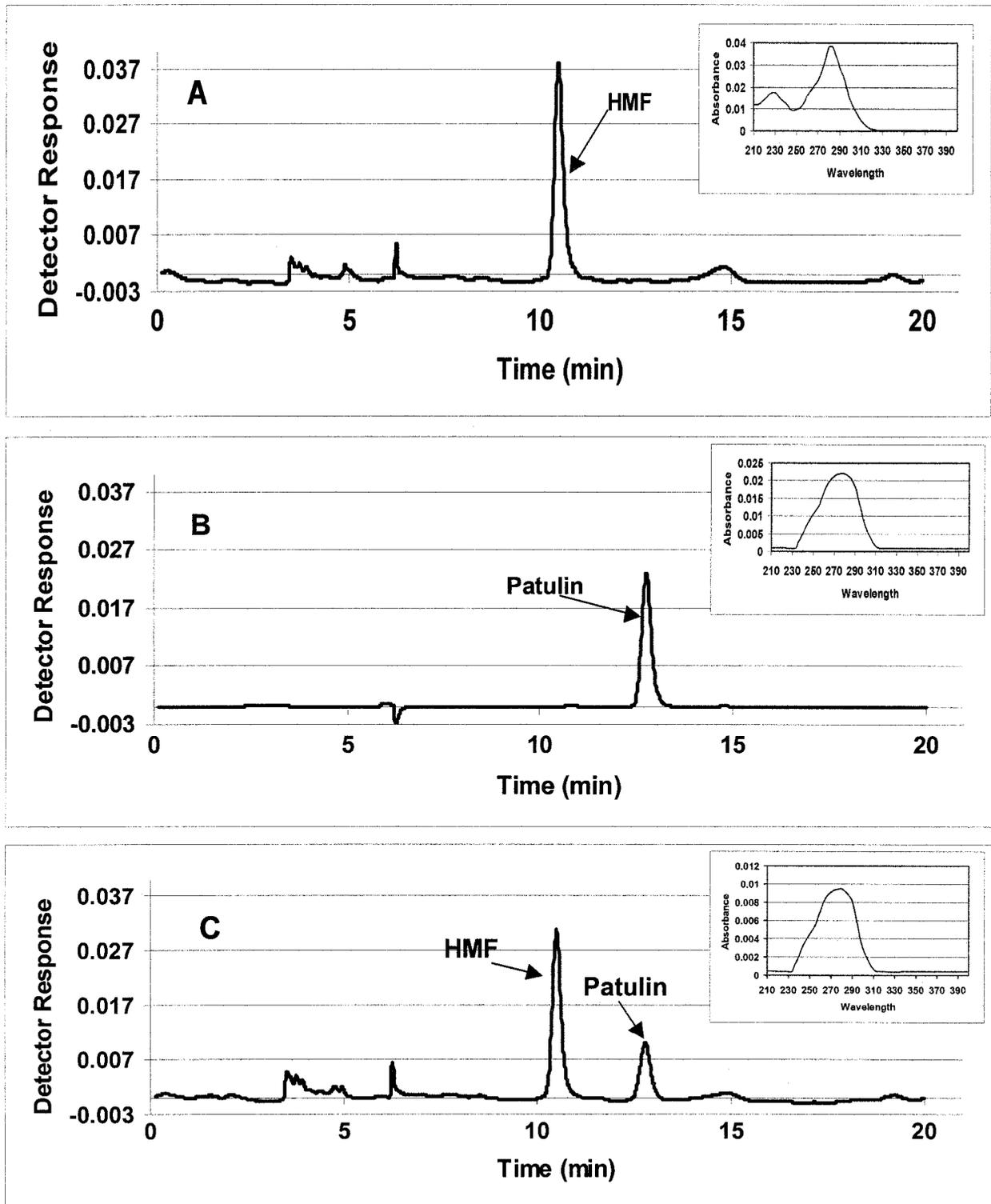
FIGURE 1. *HPLC chromatograms obtained with the use of UV detection at 276 nm for patulin and HMF in standards and in apple cider. Chromatograms A, B, and C are those for the HMF standard, the patulin standard, and an apple cider sample contaminated with HMF and patulin, respectively. Inset in chromatograms A, B, and C are UV spectra (210 to 400 nm) for the standard HMF peak, the patulin standard peak, and the suspected patulin peak, respectively.*

levels in cider pressed from fresh (nonstored) apples were determined. For the seven apple cultivars studied, no patulin was detected in cider pressed from tree-picked fruit (culled or not culled) (Table 1). In contrast, cider produced from ground-harvested fruit contained 0 to 374 μg of patulin per liter. Patulin levels for cider made from ground-

harvested Golden Delicious, Red Delicious, Granny Smith, and Fuji apples were significantly ($P < 0.05$) higher than those for cider produced from the same cultivars of tree-picked fruit (Table 1). The wide variation in patulin levels among batches of ground-harvested fruit (coefficient of variation = 13.9 to 112%) illustrates the heterogeneous distri-

TABLE 1. *Patulin contents of cider pressed from fresh (not stored) apples*

Apple variety	Tree-picked apples		Ground-harvested apples
	Culled	Not culled	
Golden Delicious	ND A	ND A	40.2 ± 7.2 B
Granny Smith	ND A	ND A	46.3 ± 13.3 B
Red Delicious	ND A	ND A	374 ± 52 B
Fuji	ND A	ND A	63.8 ± 31.9 B
Gala	ND A	ND A	ND A
MacIntosh	ND A	ND A	ND A
Red Rome	ND A	ND A	ND A

Patulin level (μg/liter) in cider pressed from[a]:

[a] Average ± standard deviation for three trials. Patulin levels were corrected for recovery. ND, not detected (below the limit of detection [1 to 3 μg/liter]). Values with different letters in the same row are significantly different ($P < 0.05$).

bution of patulin within batches of apples (Table 1). Similar results were reported by Sydenham et al. *(35, 36),* who found wide variation in patulin levels in apple juice made with apples obtained from the same lot but from different bins. These results are not unexpected, because other mycotoxins (e.g., aflatoxins and ochratoxin) are heterogeneously distributed in foods and feeds *(34, 41, 44).*

To our knowledge, this is the first report on the effects of harvesting method on patulin levels in cider. However, several publications describe the effects of culling on patulin levels in cider. Wilson and Nuovo *(45)* surveyed 100 samples of fresh cider and found that samples having the highest patulin levels were produced by cider mills that did not remove decayed apples before pressing. Similarly, Sydenham et al. *(35, 36)* reported that the removal of rotten fruit prior to pressing significantly reduced patulin levels in cider produced from apples stored at ambient temperatures for 7 to 35 days. The results of this study indicate that cider from dropped Red Delicious apples contained significantly higher levels of patulin than cider prepared from the other six apple cultivars (Table 1). No patulin was found in cider pressed from dropped Gala, MacIntosh, or Red Rome apples. These results, along with those published by Beer and Amand *(4),* Paster et al. *(28),* Wilson and Nuovo *(45),* and Spotts and Mielke *(33),* suggest that fruit cultivars differ in their susceptibilities to *P. expansum* rot and to contamination with patulin. Of the four cultivars studied by Spotts and Mielke *(33),* Royal Gala apples were found to be the most resistant to decay resulting from *P. expansum,* while Fuji apples were the least resistant. Beer and Amand *(4)* reported that the amount of patulin produced in apples artificially inoculated with *P. expansum* was inversely proportional to the firmness of the apple, an apple cultivar characteristic.

In addition to being analyzed for patulin, cider samples in this study were also analyzed for bacterium levels and yeast and mold levels as described by Merker et al. *(25).* Microbial levels showed trends similar to those of patulin levels in the samples. Aerobic plate counts and yeast and mold counts for cider from all cultivars of ground-harvested apples were nearly 2 log units higher than counts for cider produced from tree-picked fruit *(26).* The rank correlation coefficient for patulin and aerobic plate counts for the cider was 0.5120 ($P < 0.0001$), while the rank correlation coefficient for patulin and yeast and mold counts in the cider was 0.5565 ($P < 0.0001$). Although patulin was not detected in cider produced from tree-picked fruit (culled or not culled), Merker et al. *(25)* reported significantly higher microbial counts in cider pressed from unculled tree-picked apples than for cider pressed from culled fruit. Overall, the results of this study and those reported by Merker et al. *(25)* indicate that the patulin level may be a good indicator of the quality of the apples used to produce cider. These results also strongly suggest that patulin and microbial levels can be substantially reduced when tree-picked rather than ground-harvested fruit is used in cider production.

It has been recommended that juice and cider producers *(26, 39)* avoid the use of dropped apples in the production of unpasteurized cider, since several outbreaks of foodborne illness have been traced to the consumption of cider contaminated with *Escherichia coli* O157:H7, *Cryptosporidium parvum,* and *Salmonella* spp. *(2, 6, 10, 11).* Despite the strong evidence that dropped apples pose a greater risk for microbial and possibly pathogen contamination, some cider manufacturers continue to use dropped fruit in cider production. In a 1996 survey of industry practices, the U.S. Apple Association reported to the FDA that approximately 47% of the 689 survey respondents indicated that they used ground-harvested apples for the production of fresh cider *(39).* In regional surveys conducted in Virginia, Wisconsin, and New England, 32, 14, and 100% of cider processors, respectively, reported that they used ground-harvested fruit to produce cider *(6, 38, 47).* Some juice and cider processors reported that they used ground-harvested fruit in the manufacture of pasteurized or thermally treated apple products in order to avoid the risk of microbial contamination. Although thermal processing is effective in destroying microorganisms such as bacteria and yeast, patulin is resistant to the processing procedures applied to juices *(20, 43).* The stability of patulin is illustrated by the presence of the toxin in commercial apple products (juices, concentrates, jellies, baby foods, etc.) that have been thermally processed *(5, 9, 13, 27).* These findings strongly suggest that the use of dropped fruit should be avoided in the manufacture of all apple products.

Patulin levels in cider pressed from stored apples. In the United States and other countries, apples are frequently stored at cold temperatures (0 to 4°C) with or without modified atmospheres to extend the shelf life of the fruit and to provide a constant supply of raw material for cider and juice *(30).* Table 2 shows patulin levels in cider pressed from tree-picked apples that were air stored for 4 to 6 weeks at approximately 0 to 2°C. Patulin was not detected when apples were culled prior to pressing but was found in five of seven cultivars in cider pressed from unculled fruit. Patulin was not detected in cider from unculled Golden Delicious or Granny Smith apples, while patulin levels in cider pressed from unculled Gala and MacIntosh apples were >60 μg/liter.

TABLE 2. *Patulin contents of cider pressed from tree-picked apples stored at 0 to 2°C for 4 to 6 weeks*

Apple cultivar	Patulin level (μg/liter) in cider pressed from apples that were[a]:	
	Culled	Not culled
Golden Delicious	ND	ND
Granny Smith	ND	ND
Red Delicious	ND	1.0 ± 1.67
Fuji	ND	3.43 ± 31.9
Gala	ND	64.0 ± 35.4
MacIntosh	ND	60.6 ± 101.4
Red Rome	ND	12.5 ± 21.7

[a] Average ± standard deviation for three trials. Patulin levels were corrected for recovery. ND, not detected (below the limit of detection [1 to 3 μg/liter]).

TABLE 3. *Effect of culling on patulin levels in cider pressed from controlled-atmosphere–stored Fuji apples*[a]

Trial	Patulin level (μg/liter) in cider pressed from apples that were[b]:	
	Culled	Not culled
A	ND	59.9 ± 86.4
B	ND	120.5 ± 74.6
C	15.1 ± 26.2	79.9 ± 66.3

[a] Controlled-atmosphere storage conditions not known.
[b] Average ± standard deviation for at least two trials. Patulin levels were corrected for recovery. ND, not detected (below the limit of detection [1 to 3 μg/liter]).

Data shown here and data published elsewhere *(3, 4, 28)* indicate that although fungal growth is dramatically reduced at temperatures of <10°C, the growth of *P. expansum* and the production of toxin are not prevented during cold storage. Paster et al. *(28)* found that patulin levels in apples and pears inoculated with different strains of *P. expansum* generally increased as the storage temperature increased from 0 to 25°C. Beer and Amand *(4)* reported that patulin levels for MacIntosh apples stored at 4°C were substantially lower than those for fruit stored at 15 or 24°C. Fungal growth and patulin formation increased with storage time *(28, 36)*.

The effects of culling on patulin levels in cider pressed from CA-stored Fuji apples obtained from a commercial source (Table 3) were determined. In all three trials, patulin levels in cider pressed from unculled apples were >59 μg/ liter. In two trials, no patulin was detected in cider pressed from fruit that had been culled, but patulin was found at a level of 15.1 μg/liter in one trial.

Although CA conditions (gas composition, temperature, and relative humidity) were not studied here, other investigators *(18, 28, 32, 48)* have found that these factors affect *P. expansum* growth and patulin levels in fruit. Lovett et al. *(22)* reported that juice made from apples subjected to CA storage (3% O_2, 1 to 3% CO_2, 0 to 3.3°C, and >90% relative humidity) for 14 weeks contained 500 μg of patulin per liter, while juice made from air-stored apples contained 2,000 to 3,000 μg of patulin per liter. Stitton and Patterson *(32)* reported that high (>3%)–CO_2 atmospheres (with CO_2 levels higher than those used for commercially stored apples) were effective for the fungistatic treatment of stored apples. However, excessively high levels of CO_2 (>8%) negatively affected the quality of some apple cultivars. Johnson et al. *(18)* reported a lower incidence of *Penicillium* rotting in apples stored at low O_2 levels (0.75%) than in apples stored at high O_2 levels (1.0 to 1.25%).

Overall, the results presented here indicate that culling is an important technique for the reduction of patulin levels in cider made from fruit subjected to cold storage with or without modified atmospheres. Similarly, Sydenham et al. *(36)* found that the removal of damaged or rotten fruit prior

to pressing was an effective method of reducing patulin levels in cider made from apples stored at ambient temperatures. According to recent surveys, most (>91%) cider processors remove moldy, bruised, damaged, or wormy apples before these apples reach the grinder or chopper *(38, 39, 47)*. However, a substantial number (37.5%) of juice and cider producers in Virginia acknowledged that they used damaged fruit for cider production *(47)*. The results presented here indicate that stringent culling procedures are needed to keep patulin levels to a minimum in cider pressed from stored fruit.

Effects of apple-washing treatments on patulin levels in cider. Figure 2 shows the effects of different apple-washing treatments on patulin levels in cider pressed from dropped Golden Delicious apples naturally contaminated with different levels of patulin. Ground-harvested apples rather than tree-harvested apples were used in this study to ensure high initial microbial and mold counts for the apples. Washing treatments reduced patulin levels in cider by 10 to 100%, depending on the initial patulin level and the type of wash solution used. In one trial (trial B, Fig. 2), patulin levels for cider made from apples washed with a 100-ppm chlorine solution were significantly ($P < 0.05$) lower than those for cider from unwashed fruit or fruit washed with water. In contrast, trials A and C (Fig. 2) did not show chlorine solutions to be more efficacious than water in the removal of patulin from apples. No significant difference between patulin levels in ciders made from apples washed with 100- and 200-ppm chlorine solutions (trial A) was found. The extent of the removal of patulin from contaminated apples depended on the initial levels of the toxin. In trial A, in which initial levels of patulin in apples were 275 to 355 μg/liter, washing treatments reduced patulin levels by <62%. In trials B and C, in which initial patulin levels were <60 μg/liter, washing reduced patulin levels in cider by up to 100%.

These results confirm previously published reports indicating that washing treatments reduce mold counts as well as patulin levels in apples *(1, 35, 36)*. Sydenham et al. *(35)* found that patulin levels in cider decreased from 920 to 190 μg/liter after Granny Smith apples were washed with water. Similar to the results presented here, Sydenham et al.'s *(35)* results indicated that the efficacy of a water wash in re-
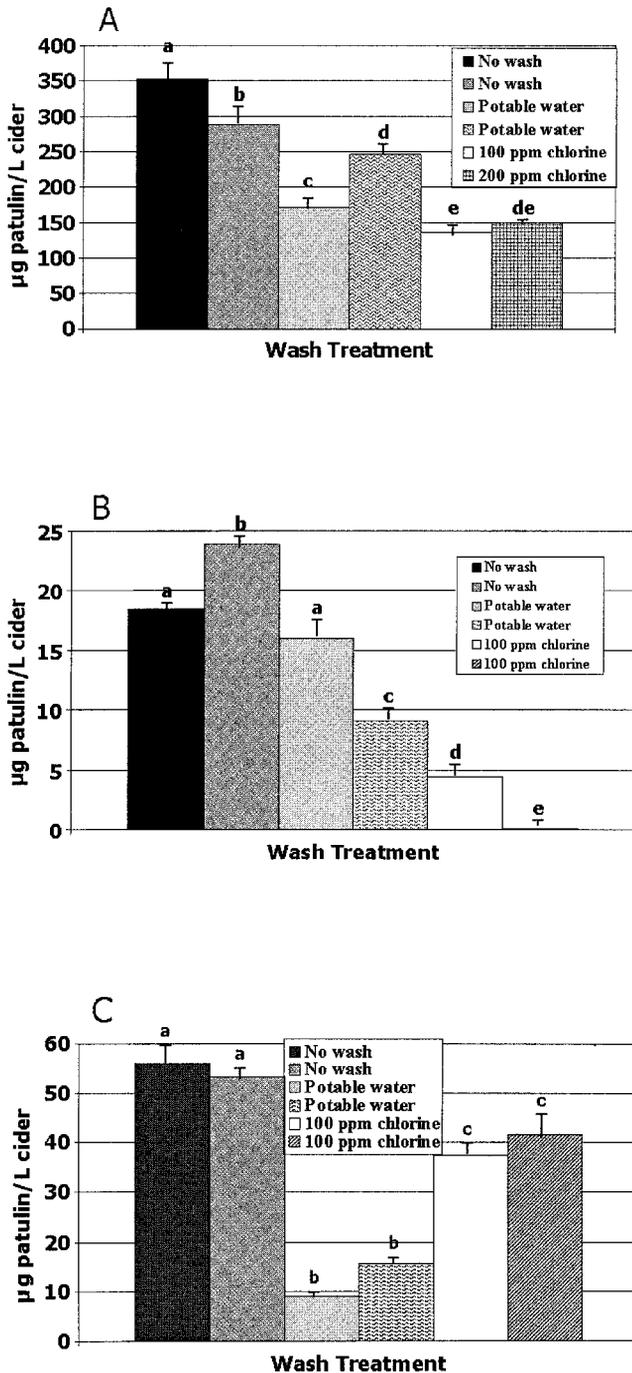
FIGURE 2. *Effects of washing treatments on patulin levels in apple cider pressed from ground-harvested Golden Delicious apples. A, B, and C refer to separate experiments involving apples with different patulin contents. Treatments include no washing, washing with potable water, and washing with 100- and 200-ppm chlorine solutions. Duplicate trials were conducted for most treatments. Each bar represents the average of three determinations. Error bars represent the standard deviation of the mean. Treatments with different letters are significantly different (P < 0.05).*

moving patulin depended on the initial patulin levels in the apples.

According to recent surveys of industry practices, the majority (>83%) of cider processors wash apples on receipt or immediately before chopping and pressing to remove soil, rot, pesticide residues, insects, microorganisms, and

other extraneous material *(38, 39, 47)*. Apples can be washed in dump tanks containing water or chlorinated water, with brusher scrubbers, and with high-pressure water sprayers *(30, 47)*. Since *P. expansum* and patulin are associated with soft rot in apples, washing may result in the removal of rotten areas of the apple and the introduction of patulin into the cleaning water *(35)*. Although there have been no reports on the effects of scrubbing treatments on patulin levels in cider, Acar et al. *(1)* reported that patulin levels were reduced by up to 54% when apples were washed with a high-pressure water spray. Total removal of patulin during wash treatments is unlikely, since patulin can diffuse up to 1 cm into the healthy tissue *(37)*. Our results and those of other investigators *(35, 37)* indicate that wash treatments alone cannot ensure that patulin levels in cider are below the 50-μg/liter action level established by the FDA and international regulatory agencies. Clearly, more work needs to be undertaken to study of the effects of wash treatment parameters (chemical additives, length of time, agitation, etc.) on the removal of patulin from contaminated apples.

Overall, the results presented in this paper indicate that the patulin level in apple cider is a good indicator of the quality of the incoming fruit and that the presence of patulin in cider or juice indicates that damaged and/or moldy fruit was used in the production of the product. The practice of using only fresh, tree-harvested apples in cider production may reduce the levels of patulin in cider. In addition, the culling of incoming fruit appears to be a good method for reducing patulin levels in cider pressed from stored (refrigerated or CA) fruit. Apple-washing treatments reduce patulin levels in cider made from contaminated fruit. However, when apples were highly contaminated with patulin, washing treatments were not able to reduce patulin levels to <50 μg/liter, the FDA action level for the toxin.

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