Disappearance of Patulin During Alcoholic Fermentation of Apple Juice

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Eight yeast strains were used in three typical American processes to ferment apple juice containing 15 mg of added patulin per liter. Patulin was reduced to less than the minimum detectable level of 50 μg/liter in all but two cases; in all cases, the level of patulin was reduced by over 99% during alcoholic fermentation. In unfermented samples of apple juice, the concentration of added patulin declined by only 10% when the juice was held for 2 weeks, a period equivalent to the time required for fermentation.

Patulin is a mycotoxin produced by a number of organisms involved in food spoilage, including *Penicillium expansum,* the cause of the blue rot storage disease of apples. This compound is toxic to higher animals (14), carcinogenic when administered subcutaneously to rats (6), and teratogenic to chicken embryos (5). Patulin has been isolated from infected apples, and the reported occurrences of patulin in commercial juice are believed to result from the use of apples with some degree of decay (15). Fermented apple cider is made from fruit similar in quality to that used for unfermented juice production. There are conflicting reports in the literature concerning the stability of patulin during the course of an alcoholic fermentation. A Canadian fermentation method resulted in little or no detectable patulin after fermentation (10). British fermentation methods have been reported to result in the removal of over 90% of the patulin during fermentation (3). However, analysis of commercial French ciders disclosed patulin in five of eight farm ciders and four of five industrial ciders (7). In the United States fermented apple ciders are produced by a wide variety of methods (1). This study was designed to evaluate the persistence of patulin in apple juice during fermentation. Eight commercial yeast strains were used in three typical American fermentation procedures.

The fermentation methods investigated were the Pacific Northwest (16, 17), California (1), and traditional (9). In the first two methods, sufficient sugar is added to yield 10 to 12% alcohol, whereas the last method relies upon the sugar present in the apple juice (about 12%) and produces a maximum of about 6% alcohol. The Pacific Northwest method maximizes the characteristic apple flavor and aroma by means of a rapid fermentation. This is achieved by the use of pure yeast cultures and allowing the fermentation to proceed until the Brix of the apple juice has dropped to 2° Brix (dissolved solids expressed as grams of sucrose in 100 g of solution). Then a total of 10% sugar is added in two equal portions during the remainder of the fermentation. The California method, in addition to being important commercially, resembles most procedures recommended for amateur home winemakers (8, 11). Pure yeast strains are used, and sufficient sugar is added at the start of the fermentation to raise the Brix value to 22°. Nutrients such as $(NH_4)_2SO_4$ or $(NH_4)_2HPO_4$, to serve as a nitrogen source, can further accelerate the fermentation of juice deficient in natural nitrogenous compounds (11). If used, the nutrient is added, and the apple juice is inoculated and fermented completely. The traditional method relies upon the natural sugars present in the apple juice, and generally no additional sugar or nutrient material is added. This method, which is still used extensively in France (2), is not popular in the United States. In the American processes pure yeast cultures are usually recommended for the fermentation of apple juice (1), but in France wild organisms naturally associated with the fruit carry out the fermentation (4; Williams, personal communication).

Eight commercial yeast strains were examined, because marked differences in metabolic activities among different strains have been observed (1). Two yeasts, Montrachet (no. 522, Davis, Calif.) and Champagne (Institute Pasteur, Paris, France) were obtained as lyophilized powders from the Red Star Yeast Corp., Milwaukee, Wis.). The remaining yeasts, Burgundy (4123), California (4105), Muscatel (8256), Sauterne (8257), Steinberg (14284), and Wortman

(4098), were obtained from the American Type Culture Collection, Rockville, Md.). The lyophilized powders were suspended in sterilized distilled water, and measured samples were used for inoculation. The ATCC cultures were activated first on orange juice media(a cold water extract of orange serum agar [Difco Laboratories, Detroit, Mich.]) and then were transferred to pasteurized apple juice. When the cultures were growing actively in the latter, samples were used to inoculate apple juice medium. All transfers in this study used 1% inocula.

In the fermentation procedure, we used 100 ml of pasteurized whole apple juice containing 15 mg of patulin per liter and 10 mg of Dowex antifoam agent (adsorbed on 0.2 g of sucrose) per liter in 125-ml Erlenmeyer flasks fitted with fermentation traps. Fermentations were conducted at room temperature (23 to 25°C). The flasks were swirled twice daily to insure suspension of the yeast and permit observation of gas evolution. Fermentation was considered complete when gas evolution ceased, which generally occurred after 10 to 14 days of incubation. Patulin concentrations were determined by the method of Stinson et al. (12). The Brix values ranged from −2.0 to 0°, indicating that all fermentations had proceeded normally. Controls of uninoculated apple juice supplemented with patulin and Dowex antifoam agent and stored under similar conditions were protected from bacterial contamination by the addition of 25% ethanol. The concentration of patulin in the controls declined by 10% in 2 weeks. Altogether, four series of fermentations were conducted. The Pacific Northwest and traditional fermentation methods were conducted as described above without additional nutrients. The California method was examined both with and without an additional nutrient [0.1% $(NH_4)_2HPO_4$]. The addition had no discernable effect on the time required for complete fermentation or disappearance of patulin.

Added patulin (15 mg/liter) was completely destroyed by all yeast strains examined during fermentation of apple juice to apple wine by both the Pacific Northwest and the California procedures. Patulin was completely destroyed during fermentation to hard cider by six of the eight yeast strains. Traces of patulin, 50 and 70 μg/liter, were found in the hard ciders produced with the California (ATCC 4105) and Steinberg (ATCC 14284) strains of wine yeasts, respectively. In these two samples, fermentation removed over 99% of the original patulin. These levels of patulin were lower than the concentrations reported in the French ciders (7); of 13 French ciders examined, 4 contained no patulin, 6 contained 100 μg/liter, and 3 contained 300 μg/liter. The French ciders contained more patulin after fermentation than did most apple juice made by the American processes. A market survey in the United States of 136 consumer packs has shown that 37% of the apple juice samples contain patulin in concentrations ranging from 40 to 270 μg/liter, with an isolated sample at 440 μg/liter (13). Fermentation of any of these samples by American methods, with elimination of more than 99% of the patulin content, would result in fermented apple ciders with undetectable patulin levels.

There are at least four factors that could account for the presence of patulin in the French-style ciders (Williams, personal communication). (i) In France cider apples are commonly harvested after they have fallen, to achieve maximum sweetness; this would expose the apples to infection by the ubiquitous *Penicillium expansum,* which invades through wounds and results in patulin production. (ii) Pulp is stored up to 12 h before being pressed and fermented, which would permit further fungal growth. (iii) Fermentations are usually with wild yeast. As these are often alcohol intolerant, arrested fermentations are common. (iv) Cider that is considered too dry is often blended with unfermented juice. Any one of these factors may contribute to the relatively high patulin content of French-style ciders. None of the above practices is followed in the United States.

Disappearance of patulin during alcoholic fermentation does not necessarily eliminate the potential health hazard posed by its initial presence. Recent work (Stinson et al., J. Food Sci., in press) has demonstrated that patulin is converted by alcohol fermentation to nonvolatile and water soluble substances. These substances that remain in the fermented product have not yet been chemically or toxicologically characterized.

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