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Review Article

Risks of Patulin and Its Removal Procedures: A Review

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ABSTRACT

Patulin is the secondary metabolite of some spoilage fungi. Despite efforts to prevent and reduce the mycotoxin, it is considered as a major problem in human health, especially in developing countries. Using different methods before and after processing of food can affect the level of patulin in the final product. The results showed that the different stages of production such as filtration, heating process, clarification or additional steps such as using radiation and absorbent materials can reduce patulin to some extent. The reduction of patulin in various processes depends on the composition of foods. In some studies, the amount of patulin after processing did not decrease sufficiently. Data showed that the use of various physical, chemical, and biological methods can reduce the patulin. However, because of patulin's thermal resistance and combination effect of food ingredients, the most effective ways to reduce patulin are using good agricultural practices (GAP) and good manufacture production (GMP) during food production.

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Introduction

Patulin is a secondary metabolite of some food spoilage fungi (1). It was firstly introduced as an effective antibiotic on 75 species of gram positive and negative bacteria (2). Patulin is a colorless crystalline compound with molecular weight of 154.12 Daltons. Patulin is present in rotten tissues and can penetrate to the surrounding tissues up to distance of about 1 cm (3, 4). It dissolves in water, ethanol, and methanol and is resistant to thermal and acidic conditions. However, it is unstable in pH>5.5 (5, 6). The resistance of patulin in apple juice is due to acidic conditions and the presence of compounds such as malic acid, serine, and threonine. The presence of this toxin can indicate that molded fruits were used as raw material for production of juices and concentrates (7). Therefore, patulin is a quality control indicator of fruit juices and concentrates.

Fungi and Conditions of Patulin Production

Patulin is produced by about 60 fungi species belonging to more than 30 genera. *Aspergillus clavatus, Aspergillus giganteus, Aspergillus terreus, Penicillium urticae, Penicillium expansum*, and *Byssochlamys nivea* are the most important fungi producing patulin (2). Production of patulin in food depends on some environmental and internal factors such as temperature, a_w, pH, gas composition of packaging, and presence of chemical preservatives (8). *Penicillium expansum* produces patulin at 0-30 °C. It is also psychrotrophic and able to grow and produce patulin at refrigerated temperatures. Moreover, apple varieties are an effective factor on the production of patulin by *Penicillium expansum*. *Byssochlamys nivea* grows rapidly at 30-37 °C. However, the highest toxin production is at 21 °C (2). *Penicillium expansum* and *Aspergillus spp.* are major patulin producing fungi before food processing. While *Byssochlamys* genus is known as the most mycotoxins producing genus after the pasteurization process due to its high thermal resistance. Atmosphere gas composition is an effective factor on the amount of patulin production by *Penicillium expansum*. It has been reported that the toxin production rate was 2.5 micrograms per milliliter at low temperatures. However, the production of patulin was 0.5 micrograms per milliliter in the modified atmosphere with 96% nitrogen, 3% oxygen and 1% carbon dioxide, and 90% relative humidity at 5 °C (9).

Sources of Patulin

Patulin can be found in many fruits and vegetables such as apples, grapes, peaches, nectarines, apricots, strawberries, tomatoes and olives. It has also been isolated from cheddar cheese, cereal, and cereal products such as wheat, barley, flour, and bread. The most common sources of patulin are apple and its products. It should be noted that no patulin is produced in intact fruits (10, 11).

Regulatory Limits for Patulin

The Europe Union and the World Health Organization (WHO) have set the maximum acceptable level of patulin to be 50 µg/kg for fruit juices, wine and fermented beverages derived from apples and 25 µg/kg for solid apple products. The temporary acceptable daily intake of ptulin is 0.4 µg/kg in apple products consumed by children (12).

Risks of Patulin

The International Agency for Research on Cancer (IARC) has categorized patulin in Group 3 carcinogenic. This means that there is no adequate information regarding the carcinogenicity of patulin in humans and animals (13). Patulin received from fruit drinks is more toxic than solid foods. It may be related to patulin binding to food components and reduction of its absorption (14). Primarily, patulin has toxicity effect on plasma membrane and it can change the activity of different enzymes. The rupture of the plasma membrane occurred due to reaction of patulin with sulfide groups, protein and amino acids (8, 15). Patulin can cause acute symptoms (jitteriness, seizures, lung congestion, edema, hyperemia, and expansion digestive tract, especially in the gastrointestinal tract) and chronic symptoms (hemorrhagic lesions such as genotoxic, nephrotoxic, immunotoxic symptoms and synthesis inhibition of protein, RNA, and DNA). This mycotoxincan cause pathological changes including brain edema, bleeding lungs, atrophy of the capillary vessels in the liver, spleen and kidney (4, 16, 17).

Before Food Processing

1. Pre-Harvest

Droughts, rainfall, humidity at the cluster time of plants and contamination with insect could increase the formation of mycotoxins. Patulin represents resistance to different processing of fruit juices. Therefore, good agricultural practices (GAP) are an important and effective way to prevent the formation of the mycotoxin before processing (18). Application of fungicides and calcium or phosphorus fertilizers could improve the texture resistance to rot. Fungus-resistant plant varieties can be produced by genetic engineering.

2. Post-Harvest

Harvesting should be done in less than 18 hours when the fruit is maturated. Cooling of the product and eliminating waste could be an effective factor for reducing patulin (9). Harvesting exposes fruits to mechanical damage and fungi growth. Transportation should be done accurately and in appropriate and clean containers. Antioxidants (such as diphenylamine) and fungicides (such as benomyl and benzylmidazoles) should be used before storage. The products should be protected from moisture, insects, and other harmful environmental factors and stored on clean and dry surfaces (18, 19).

During Food Processing

1. Physical Methods

Physical, chemical, and biological methods can also be effective during and after processing (9). It has been reported that application of physical methods including mechanical separation, washing, inactivating by heat, radiation (microwaves), ultrasound, ultra violet, solvent extraction, and the like are successful techniques for patulin reduction. Heating, radiation, absorption and solvent extraction methods are expensive and may cause damage or nutrient loss. Furthermore, patulin is resistant to heat (20). In food processing, washing and removing the decayed part are low-cost methods for reducing patulin (21). Eskandari and colleagues examined the effect of different processing stages of commercial fruit leather on patulin reduction. The maximum reduction of patulin was 24.60% and 18.20% in the formulation and concentration processes, respectively. The amount of patulin reduction after drying, filtration, and basic cooking stages was 8.58%, 3.28%, and 2.48%, respectively. The researchers found that different production stages could not reduce patulin in the raw material to an acceptable level. The maximum reduction of patulin was seen in the formulation stage. Addition of other materials or ascorbic acid and using heat in the formulation stage were responsible for this reduction. Patulin decreased to 3.82% at the filtration stage. Despite the separation of the skin, due to patulin solubility in water, a large amount of patulin is penetrated into apple pulp and juices and enters the next step of the process (6). The amount of patulin reduction in apple juice after the pasteurization process, enzyme treatment, microfiltration, and evaporation were 39.6, 28.3, 20.1 and 28.4%, respectively (3). In the latter study, a greater reduction in patulin level was seen in the filtration stage. In the production of apple juice, compared with apple leather, smaller orifice filters are used and particulate matter and pulp are also removed. In another study, high-pressure washing reduce patulin up to 54%. Four different processes including bentonite application for finning, treatment with pectinase, filtration, and centrifugation were also investigated. Reduction of patulin after pectinase treatment and filtration were 28.3% and 20.1%, respectively. Centrifugation was the most effective way to reduce patulin up to 89% (7).

In the process of apple juice production, reduction of patulin after thermal treatment and filtration with activated charcoal was 13.4% and 22.9%, respectively. In the thermal process, a small amount of patulin was reduced due to thermal resistance in acidic conditions (22). In order to ensure the removal of heat-resistant fungi such as ascosporic and patulin, filtration can be a critical control point in the production process of apple juice (9). Acar and co-workers reported that 25% and 39% of patulin reduced after using conventional filtration and ultrafiltration (23). The average patulin reduction during evaporation (80 °C for 20 mins) was 14.1 %. Moreover, the amount of patulin at 96 °C for 36 seconds can be reduced up to 28.4% (7).

Wheeler and colleagues reported that patulin reduction reached 18% at 90 °C for 10 seconds (24). Doyle and co-workers also found patulin decreased by 90% at 105 °C for 29 seconds (25). Different thermal stability of patulin is related to its reaction with food ingredients especially ascorbic acid. Alcoholic fermentation, the addition of vitamins, irradiation process, and use of active carbon and sulfur dioxide could reduce the thermal stability of patulin. Irradiation is a physical method for patulin destruction. Tikekar and colleagues examined the ultraviolet (UV) effect on patulin reduction in clear and cloudy juices. They found that UV light could destroy patulin in clear fruit juice more than cloudy ones. Suspended particles and phenolic compounds in cloudy juice prevent contact between patulin molecules and waves. UV with amount of 5.6 J/cm^2 after than 20 passes, reduced patulin in apple juice and cider up to 30% and 89% respectively. The addition of fructose and juice filtration before using UV treatment could increase patulin destruction (20). After UV exposure at 253.7 mJ/cm, 56.5%, 87.5%, 94.8% and 98.6% reduction of patulin can be achieved, in the model solution, apple cider, and apple juice without ascorbic acid addition and apple juice with ascorbic acid addition, respectively (5).

The maximum reduction of patulin has been seen in apple juice with ascorbic acid, so ascorbic acid is considered as one of the effective factors in reducing of patulin (5). Dong and colleagues studied the effect of UV on reduction of patulin. They found that the greatest reduction of patulin was achieved after 15 seconds of UV exposure of 99.4 mJ/cm (4). In unfiltered apple cider, patulin levels decreased by 43.4% while quantifiable changes in the chemical composition or organoleptic properties of cider were not seen (4).The effect of gamma rays on patulin reduction was investigated in apple juice model system and the researchers found that it was decomposed in a dose 4 KGy of gamma radiation. But in other juices, patulin was relatively stable due to the presence of compounds such as lactic acid, ascorbic acid, and histidine. The amount of these compounds in apples is negligible (7). Fathi Achachlouei and colleagues treated apple juice containing patulin with different types of activated charcoal. Activated charcoal powder had more ability to absorb apple juice patulin than granular charcoal form and consumption of 5 gr/L of activated charcoal powder could remove patulin completely (26). In another study, the use of activated charcoal with a density of 10 and 20 mg per liters reduced patulin in apple cider up to 93.3% and 100%, respectively. Hydroxyl methyl furfural (HMF) was reduced significantly as negative factor on apple quality (27).

2. Chemical Methods

Chemicals used to remove mycotoxins are divided into acids, bases, oxidizing and reducing factor, salt, and chlorinated materials. Patulin reduced by 42% in the presence of 100 mg/kg SO_2. Also 100 ppm sulfur dioxide reduced patulin up to 50% (9). The reduction of patulin in samples containing ascorbic acid was 30% after storage for 31 days and patulin reduction was 68% to 71% in samples without ascorbic acid (28). In another study, adding 2% of ascorbic acid to phosphate buffer solution removed patulin immediately. The reduction of patulin in apple juice containing 5% ascorbic acid was also more than control samples. However, compared with phosphate buffer solution, the rate of decline was lower (29). Radicals produced by oxidation of ascorbic acid destroy patulin. When ascorbic acid was oxidized completely, destruction of patulin would not increase. Storage conditions (light, oxygen and metal ions) affect the stability of patulin in the presence of ascorbic acid. In the absence of oxygen (such as in a bottle) or metal counteractive agents, patulin is stable in the presence of ascorbic acid (5, 9). Vitamin B group such as thiamine hydrochloride, pyridoxine hydrochloride, and calcium-d-pantothenate reduced patulin up to 55.5-67.7% after 6 months at 4 °C (30).

3. Biological Methods

Biological detoxification of mycotoxins includes their absorption by absorbent material and inactivation by microorganisms and specific enzymes. In the enzymatic method, mycotoxin is restructured or has been decomposed. These compounds are non-toxic products or less toxic. Application of yeast as biological control is known as one way of reducing the use of synthetic fungicides (31). During fermentation by yeast, patulin can be reduced up to 90%. Yuan and co-workers studied the reduction of patulin in apple juice by 12 inactive species of *Alicyclo bacillus*. They found that *A. acidoterrestris* 92 and *A. acidoterrestris* 96 had the greatest impact on reducing patulin. The greatest reduction of patulin after 24 hours' incubation with these micro-organisms was 88.8% and 81.6%, respectively. They had no negative effects on the quality parameters of apples (32). In a similar study, the reduction of patulin in apple juice by 10 species of inactive yeast was investigated. The researchers found that 8 of 10 the species could reduce patulin up to 50% after 24 hours. Quality parameters of apple juice such as Brix, sugar content, titratable acidity and color did not change (33).

Pichia ohmeri 158 yeast could also reduce the levels of patulin at 25 °C up to 83%and 99% after 2 and 5 days, respectively. Reduction of patulin by *pichia ohmeri* 158 is an active process and is due to the metabolism of the yeast. *Pichia ohmeri* did not change quality parameters of fruit juices such as weight, fruit firmness, ascorbic acid, and acidity (34). *Niger Rhizopus* and *Aspergillus* fumigates were able to destroy patulin through the same mechanism (1).

Conclusion

Various stages of production such as filtration, heating process, clarification or additional steps such as using radiation and absorbing materials can reduce patulin level in final product. These methods are expensive. Due to thermal resistance of patulin, it does not change mostly in condensation and pasteurization steps and remains in final products. Application of UV in filtered products reduces patulin without significant change of quality parameters. But, in unfiltered products, the effective dose for considerable reduction of patulin level is more than filtered products that can cause undesirable changes in fruit juice quality. Studies have shown that patulin is resistant to various stages of production. Therefore, the most effective way to reduce patulin in the final products is implementation of GAP in farms or GMP implementation during food processing steps.

Conflict of Interest

None declared.

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