

Review

# Patulin removal in fruit-based products using probiotics and potential probiotics

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## CITATION

Zoghi A. Patulin removal in fruit-based products using probiotics and potential probiotics. Food Nutrition Chemistry. 2024; 2(2): 276.  
<https://doi.org/10.18686/fnc276>

## ARTICLE INFO

Received: 17 February 2024  
Accepted: 28 March 2024  
Available online: 8 April 2024

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**Abstract:** Patulin (PAT) is a prevalent mycotoxin frequently found in fruit and its derivatives, such as apple, pear, and juices. Despite worldwide attempts to diminish the levels of PAT at every stage of the fruit production process, its contamination rate remains high. This mycotoxin is worrisome due to its potential adverse impacts on human health. Eating PAT-contaminated fruit can lead to acute and chronic health issues. It is established, by the Joint FAO/WHO, a maximum tolerable daily intake for PAT at 0.4 µg/kg/day. Therefore, monitoring for PAT contamination is essential for the safe consumption of fruits and fruit-related products such as juices, purees, ciders, jams, marmalades, vinegar, and dried fruits. PAT has physiochemical properties that enable its survival in cold, hypoxic, acidic, or high-temperature conditions. Ideally, detoxification procedures should aim to reduce the level of toxins to safe levels whilst preserving the nutritional and palatable values of the treated commodity. There are several physical, chemical, and biological techniques available for PAT detoxification. However, while physical and chemical methods can remove PAT, they may also lower the nutrient quality and organoleptic properties of the food. Biological detoxification is an effective, environmentally friendly, easy, and cost-effective method, as established by existing research. It has proven efficacy in food safety research and regulatory compliance programs. Probiotics have been studied for their potential to reduce PAT in foods via different mechanisms (such as adsorption, degradation, and transformation), as well as all their health-beneficial effects. In this review, the reduction of PAT in fruit-based products using probiotics or potential probiotics is widely discussed.

**Keywords:** mycotoxin; lactic acid bacteria; detoxification; decontamination; bioremoval

## 1. Introduction

Mycotoxin contamination poses a significant threat to consumer safety and agricultural production [1]. Certain fungi produce toxic secondary metabolites known as mycotoxins, which can contaminate numerous food commodities, including fruits and their derived products [2]. The primary mycotoxin present in fruits and their derived products is patulin (PAT). PAT, an unsaturated heterocyclic lactone (4-hydroxy-4H-furo[3,2-c] pyran-2(6H)-one), is a toxic secondary metabolite produced by various fungal species in the *Penicillium*, *Byssoclamy*, and *Aspergillus* genera [3]. *Penicillium expansum* is likely the most common pre-harvest and postharvest fruit contaminant, while *Byssoclamy nivea* is the most heat resistant among known producing species and a potential producer of PAT in pasteurized fruit juices [4]. Additionally, the fruit's physical and chemical characteristics, including strength, skin thickness, flesh firmness, pH, sugar content, and presence of antimicrobial compounds, also influence PAT formation [5].

Fruit plays a crucial role in human nutrition and is a staple in the human diet. Fruits of subpar quality, stored in rooms with or without controlled atmospheres, are utilized to make juices, compotes, purees, concentrates, ciders, and dehydrated (dried) products. This results in products that are highly contaminated with PAT [6]. Fruits are rich in water and sugar, which enhances PAT activity [7]. PAT is stable at low pH levels around 4 and is synthesized in ripened fruits. Concerns about food safety problems associated with PAT contamination have increased globally due to reports of food safety issues [5]. The presence of PAT in baby food, such as fruit juices, is a significant concern.

Different food processing methods can impact the level of PAT in the final product. The previous studies revealed that filtration, heating, and clarification during various stages of production can reduce PAT levels to a certain extent. Nevertheless, due to the thermal resistance of PAT and the combined effect of product ingredients, the PAT amount does not decrease sufficiently after these processes [8]. There are various physical techniques (thermal treatment, irradiation, high-pressure processing, ultrasonic treatment, and cold plasma treatment) and chemical methods (sodium hypochlorite treatment, sodium bisulfite treatment, ozone treatment, hydrogen peroxide treatment, and ultraviolet treatment) available to decrease PAT levels in fruits and their derivatives [5]. However, these approaches have registered some drawbacks, including safety issues, potential nutrient loss, chemical risks, restricted effectiveness, and elevated expenses [9]. There has been a recent surge of interest in the use of biological methods to eliminate PAT from fruit products.

Efficient elimination of PAT from fruit products by means of bacteria, yeasts, and their derivatives or fluid residues have been documented by several researchers [10,11]. Lactic acid bacteria (LAB) are even more beneficial in the eradication of PAT and are commonly utilized as a probiotic for humans [12]. Probiotics are generally recognized as safe (GRAS). "Probiotics are live microorganisms that demonstrate numerous health benefits when consumed in adequate amounts", as explained by FAO (Food and Agriculture Organization) [13]. These benefits include the elimination of lactose intolerance, prevention of diarrhea, support for immunity, cholesterol reduction, inhibition of colon cancer, and inhibition of intestinal and gut pathogens, as well as anticarcinogenic and antimutagenic effects [14]. Probiotics can eliminate PAT from foodstuffs through the mechanisms of using live or dead microorganisms or their specific enzymes. The ability of non-viable bacteria to detoxify is critical as their viability may be reduced through digestion [2]. Certain probiotics that can adhere to intestinal cells can rapidly pass through the gastrointestinal tract when combined with PAT [15]. In other words, if probiotics are inserted into a contaminated fruit juice and adsorb PAT, probiotic-PAT complexes are provided in the juice. After consumption of this probiotic fruit juice, the probiotic-PAT complexes do not adhere to intestinal cells and pass through the GIT (Gastrointestinal Tract). Therefore, PAT which is binned to probiotics, passes the GIT with probiotics and does not have adverse effects for consumers.

This work is a review of the literature on the removal of patulin in fruits and fruit-based products using probiotics or potential probiotics. Also, factors affecting the PAT bioremoval process and the mechanism of this phenomenon are widely discussed.

## 2. Search strategy

This section describes the methodology used to select the literature for review. For the literature review, standard search strategies were employed by querying the available online databases (Scopus, PubMed, Science Direct, ISI Web of Knowledge, and Google Scholar), between the years 2010–2023, by using terms including “Detoxification”, “Patulin”, “Probiotics”, “Bioremoval”, “Mycotoxin”, “Adsorption”, “Lactic acid bacteria”, “Yeast”, “Food safety”, and “Fruit-based products”. The reference lists of each article have been reviewed in detail to find additional articles. The selected literature was categorized according to patulin detoxification in fruit-based products using different strains of probiotics/potential probiotics and was reviewed independently in full text.

## 3. Synthesis of PAT

PAT biosynthesis comprises enzymatic reactions within fungal cells. Initially, the polyketide synthase enzyme catalyzes the condensation of two acetyl-CoA molecules, resulting in 6-methyl salicylic acid. Subsequently, PAT is created from the 6-methyl salicylic acid via a sequence of oxidation, decarboxylation, and esterification reactions. PAT is a secondary metabolite produced from polyacetate. Its metabolic pathway has been extensively studied using cell-free extracts and kinetic pulse-radiolabelling systems. In the next stage of PAT biosynthesis, 6-methyl salicylic acid decarboxylase activity converts 6-methyl salicylic acid into m-cresol. Later, m-cresol 2-hydroxylase converts m-cresol to m-hydroxy benzyl alcohol. There is a current debate about the following step in the biosynthesis of PAT, with two suggested mechanisms. Nonetheless, both mechanisms affirm that m-hydroxy benzyl alcohol gradually converts to gentisaldehyde. Thus, the transformation of m-hydroxy benzyl alcohol to gentisaldehyde is a crucial stage in the PAT biosynthetic pathway. Moreover, different environmental factors like pH, temperature, and nutrient availability can affect PAT production in fungal cells [5].

## 4. Adverse health effects of PAT

PAT has been linked with immediate symptoms of toxicity, including nausea, vomiting, and diarrhea. Additionally, PAT can cause immunotoxicity and genotoxicity, which can result in long-term health effects [16]. Furthermore, the International Agency for Research on Cancer denotes PAT as a Group 3 substance, implying that it may have the potential to cause cancer, although there is currently limited evidence to support its carcinogenicity [17]. It is noteworthy that PAT can undergo degradation when subjected to food processing or storage, thereby leading to the formation of other harmful compounds. Furthermore, PAT may undergo interaction with other compounds present in food, which can have an impact on its bioavailability and toxicity [18]. PAT appears to pose a significant hazard during the postharvest life of fruits, from single grains to the contamination of whole fruit, ultimately resulting in the spoilage of the entire stored fruit [19].

The ingestion of PAT may result in various adverse outcomes, such as agitation, dyspnoea, pulmonary congestion, convulsions, edema, hyperemia, ulceration,

gastrointestinal tract distension, epithelial cell degeneration, intestinal hemorrhage, vomiting, inflammation of the intestines, and harm to the gastrointestinal and renal tissues. Furthermore, chronic PAT consumption has been connected with a range of health hazards, including neurotoxicity, immunosuppression, immunotoxins, teratogenicity, genotoxicity, and carcinogenicity. In animal studies, high levels of PAT exposure have been linked to liver damage and immunotoxicity [20].

PAT has been demonstrated to cause cell effects including disruption of the plasma membrane, and inhibition of protein synthesis of Na<sup>+</sup> coupled amino acids, transcription, translation, and DNA. Additionally, it inhibits the production of interferon from T-helper type 1 cells [21,22]. PAT is toxic to a number of enzymes with a sulfhydryl group in their active site [23]. Also, PAT has been found to facilitate intramolecular and intermolecular protein cross-linking, favoring cysteine's thiol group but also affecting the side chains and amino groups of lysine and histidine. This process promotes the formation of cross-links between amino acids within and between proteins [24].

## **5. Detection analysis of PAT in fruit products**

The main focus of managing PAT contaminations is developing reliable and sensitive assays for detecting PAT in various food matrices. To identify and quantify PAT in food, several methods such as thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography with ultraviolet detection (HPLC-UV) can be used. The use of high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS), capillary electrophoresis (CE), fluorescence polarization, chemiluminescence assays, quantitative PCR assays, surface plasmon resonance (SPR), quartz-crystal microbalance (QCM), electrochemical reduction techniques, and so on, is prevalent in many scientific studies [25].

For several years, the primary technique for identifying PAT involved a process of solvent extraction, then cleaning, and finally chromatographic analysis with HPLC-UV detection at 277 nm. This methodology is flawed due to the presence of substances that interfere with fruit processing [26]. While liquid chromatography-mass spectrometry (LC-MS) possesses impressive separation and identification capabilities, the physicochemical properties of patulin pose a challenge to this method. PAT is a hydrophilic and highly polar polyketide, with a monoisotopic molecular mass of 154.0266 Da, consistent with 13 fungal secondary metabolites, including intermediates from PAT biosynthesis pathways like neopatulin. Neopatulin and PAT are chemically similar substances, but neopatulin is an optically inactive isomer, making it challenging to differentiate through mass spectrometry analysis [27]. Continual advancements are being made in PAT detection methods, with one notable example being the use of a near-infrared (NIR) technique in combination with fluorescently conjugated anti-PAT antibodies which can identify quantities as low as 0.06 µg/L in diluted apple juice [28].

## 6. The worldwide incidence of PAT in fruit-based products

PAT contamination is widespread in fruit-based products, particularly those made from apples. The Food and Drug Administration (FDA) has set guidance levels for PAT, which are 50 µg/kg for apple juice and apple juice concentrate, 25 µg/kg for solid apple products, and 10 µg/kg for apple-based products intended for infants, in line with the maximum limits established by the European Union and World Health Organization (WHO) [5]. The Joint FAO/WHO Food Standard Program, CODEX (Codex Alimentarius Commission) Committee on Contaminants in Food, has established a provisional maximum tolerable daily intake (PMTDI) for PAT at 0.4 µg/kg/day [7]. Various surveys have been conducted worldwide to determine the levels of PAT contamination in fruits and fruit juice concentrates. The occurrence of PAT in fruits and fruit-based products around the world, in the last 10 years, is summarized in **Table 1**.

**Table 1.** Worldwide natural occurrence of patulin in fruit-based products (recent 10 years).

Country	Fruit products	Total positive samples %	Analytical method	LOD µg/L	Amount of patulin µg/L	Reference
Serbia	Fruit juices	51.40	HPLC-UV	2.1	65.4	[29]
Romania	Apple juice	6	HPLC	0.7	101.9	[30]
Tunisia	Apple juice; Mixed juice; Pear juice; Concentrated juice	64.28; 50; 47.6; 80	HPLC-UV	-	122.3; 55.7; 231; 889	[31]
Qatar	Apple juice	100	LC-MS	5.27	82.2	[32]
India	Commercial apple products	18	HPLC-UV	7.5	112.2	[33]
Argentina	Pear marmalade	16.66	LC-MS	-	44676	[34]
Thailand	Grape	38.88	LC-MS	-	3.5	[35]
Malaysia	Lychee juice	5.88	HPLC-UV	0.25	13	[36]
China	Apple juice	100	HPLC-DAD	6.30	78	[37]
China	Dried longans; Dried figs	90.4; 61.9	HPLC-UV	7.5	194.3; 278.9	[38]
Iran	Apple Juice	100	HPLC	6.0	173	[39]
Portugal	Apple juice; Tomato products	44.4; 35.7	GC-MS	5.7	45.77; 47.72	[40]
Japan	Apple Juice	-	HPLC-UV	0.5	464	[21]
Pakistan	Apple; Grape	57.4	HPLC-UV	0.04	270; 466	[41]
Czech Republic	Apple; Pear; Fruit juice mix	-	LC-MS; HPLC-UV	0.5; 0.01; 3.5	122; 3156	[42]

LOD: Limit of detection; HPLC-UV: High-performance liquid chromatography with ultraviolet detection; LC-MS: Liquid chromatography-mass spectrometry; HPLC-DAD: High-performance liquid chromatography with a diode-array detector; GC-MS: Gas chromatography-mass spectrometry.

## 7. Bioremoval activity of probiotics and potential probiotics

*Lactobacillus (L.)*, *Bacillus*, *Bifidobacterium (B.)*, *Enterococcus (E.)*, and *Saccharomyces (S.) cerevisiae* are the most prominent probiotics that are capable of PAT removal in foodstuffs [43]. **Table 2** shows some of the recent research, in which probiotics or potential probiotics have been used for PAT removal in fruit-based products.

**Table 2.** Recent researches regarding probiotic and potential probiotic strains application for patulin bioremoval in fruit-based products.

Fruit source	Microorganism	Bio-removal range (%)	Mechanism of decontamination	Remarks	Reference
Apple juice	<i>L. pentosus</i> DSM 20314	53.14	Degradation	24 h, initial PAT content 500 µg/L	[44]
Apple juice	<i>L. kefiranofacien</i> JKSP109	93	Adsorption	37 °C, 24 h, initial PAT content 100 µg/L	[45]
Liquid medium	<i>B. animalis</i> VM 12	80	Adsorption	pH = 5	[46]
Apple juice	<i>Bacillus subtilis</i> CICC 10034	10	Degradation	24 h, 25 °C	[47]
Apple products	<i>L. plantarum</i>	100	Degradation to E-ascladiol, Z-ascladiol, and hydroascladiol	37 °C, 4 h	[48]
Aqueous solution	<i>L. brevis</i> LB-20023	35	Binding by polysaccharides and proteins from cell wall	37 °C, 48 h	[49]
Apple juice	<i>L. plantarum</i> 13 M5	43.8	Degradation to E-ascladiol	Initial PAT content 5 mg/L	[50]
Aqueous solution	<i>E. Faecium</i> EF031	50	Binding	37 °C, 48 h	[51]
Apple juice	<i>S. cerevisiae</i> ATCC 204508	100	Degradation to E-ascladiol and Z-ascladiol	30 °C, 110 h	[52]
Apple juice	<i>L. plantarum</i> ATCC 8014	95.91	Adsorption	4 °C, 6 weeks	[6,53]
Apple juice	<i>L. plantarum</i> ATCC 8014 (NaOH-treated)	59.74	Adsorption	4 °C, 48 h	[54]
Apple and pear juice	<i>L. casei</i> YZU01	95	Degradation	36 h, initial PAT content 10 µg/mL	[55]
Apple juice	<i>B. bifidum</i>	56	Adsorption	37 °C, 24 h	[56]

Abbreviations: *Lactobacillus*: *L.*; *Saccharomyces*: *S.*; *Bifidobacterium*: *B.*; *Enterococcus*: *E.*; Patulin: PAT.

Hatab and colleagues [56] conducted an experiment to determine the removal yield of PAT by *Lactococcus lactis*, *L. rhamnosus*, *B. bifidum*, and *B. animalis* (both viable and dead) at 37 °C for 24 h. The results demonstrated that the most significant removal was observed with unviable bacteria; specifically, *B. bifidum* (56%), *L. rhamnosus* (52%), *Lactococcus lactis* (36%), and *B. animalis* (21%). The efficiency of *L. rhamnosus*, *L. helveticus*, *B. bifidum*, and *B. animalis* for PAT biotransformation in apple juice (pH 4) was observed at 30 °C and 37 °C for 24 h with artificially added concentrations of 100, 150, and 200 µg/mL of PAT. The results indicate that at an initial concentration of 100 µg/mL and 30 °C, the biotransformation procedure demonstrates efficacy. Furthermore, in the presence of *L. rhamnosus*, the reduction of PAT reached approximately 82% [57]. Zoghi and colleagues [6,53] have reported that *L. acidophilus* ATCC 4356 and *L. plantarum* ATCC 8014 can eliminate PAT in apple juice. The study revealed that refrigerating apple juice with viable *L. plantarum* ( $3.6 \times 10^{11}$  CFU/mL) along with citric acid, ascorbic acid, and fructooligosaccharide resulted in a 95% reduction of PAT levels over a six-week period. The PAT elimination process was rapid on the first day and continued slowly over the course of 42 days. The mechanism of PAT bioremoval by probiotics is described below.

*L. brevis* 20023 eliminated PAT from a working solution containing 4000 µg/L at a temperature of 37 °C for 48 h, according to Wang et al. [49]. This bacterium, with a significant surface area and cell wall volume, reduced the concentration of PAT by 65%. Intriguingly, a separate study on *S. cerevisiae* reported a 100% degradation rate for PAT (50 µg/L) after two days. PAT degradation was identified as an enzymatic hydrolysis reaction in the study, despite the fact that the PAT-metabolizing enzymes were not induced through incubation with PAT [50].

## 8. Factors affecting the efficacy of PAT bioremoval

Various factors, including PAT concentration, temperature, pH, inoculum size, incubation time, and probiotic or potential probiotic species, have been identified as significant in the bioremoval process. Research by Hatab et al. [56] found that *B. animalis* 6165 showed decreased PAT binding in less acidic conditions. Topcu et al. [51] reported that nonviable cells of *E. faecium* exhibited the highest PAT adsorption at pH levels below 5, perhaps due to the impact of hydrogen bond interactions on PAT removal. Fuchs et al. [46] reported that the optimum removal of PAT occurs at pH 5. Zoghi et al. [6] stated that the PAT concentration affects the removal rate of PAT in apple juice by probiotics. Additionally, they revealed that the removal process of PAT by probiotics is prompt and begins upon direct contact. Topcu et al. [51] demonstrated that the degradation of PAT by living and non-living cells of *E. faecium* strains depended on the strain (due to the heterogeneity in bacterial cell wall compositions) and improved with incubation time (15.8% and 21% within 1 hour, and 41.6% and 45.3% within 48 h, correspondingly). Guo et al. [37] reproduced similar outcomes in an additional study, indicating higher levels of PAT elimination with prolonged incubation durations.

Pretreatment of probiotics or potential probiotics has an impact on the bioremoval process. This approach results in the denaturation of proteins, modifying the charge distribution and altering the hydrophobic surface arrangement of bacterial surfaces. Consequently, it enhances adsorption [15]. Bioremoval is increased by heat treatment processes as it facilitates adsorbing by altering the cell surface. The acid pretreatment of bacteria leads to a reduction in cell wall thickness due to the breakdown of monomers released from proteins, amide, and glycosidic linkages in peptides and polysaccharides. On the other hand, alkaline pretreatments remove coating compounds from the surface of bacteria, leading to a change in the availability of binding sites. This results in a neutralization of acidic groups and alteration of the electronegativity of the cell surface [12].

Wang et al. [49] found that PAT removal was greater in *L. brevis* inactivated cells following heating compared to viable cells. This increase in binding efficiency was due to the expansion of the cell wall and surface area caused by heat treatment. Proteins and polysaccharides were found to be involved in PAT elimination. Zoghi et al. [54] observed that pretreatment of probiotic strains with NaOH resulted in reduced PAT levels in apple juice by *L. acidophilus* ATCC 4356 and *L. plantarum* ATCC 8014. No differences were found in PAT reduction between viable and dead bacteria, regardless of whether they were treated with heat or acid. After two days of cold storage, NaOH-treated *L. plantarum* showed a higher reduction rate (61%) than

NaOH-treated *L. acidophilus* (54%). Guo et al. [37] reported that NaOH-treated *S. cerevisiae* is highly effective at removing PAT in apple juice.

## 9. Mechanisms of PAT bioremoval

Some researchers have provided insight into the mode of action for removing PAT with probiotics and potential probiotics. The primary mechanisms proposed for the biological removal of PAT are the adsorption of PAT by probiotic cells [6,53,54], degradation of PAT by microbial enzymes [11], and patulin transformation via reaction with some produced metabolites by probiotics [58].

### 9.1. Adsorption

Probiotics can form non-covalent bonds between PAT and cell surface. According to Liu et al. [59], the peptidoglycan and polysaccharides found on the surface of probiotics play a crucial role in bioremoval processes. Moreover, the functional groups present on probiotic cell walls are essential in the PAT adsorption process, as the OH and CO groups of patulin can bind to the protein surface of probiotic cell walls. Additionally, the PAT adsorption process involves the amino (-OH/-NH), polysaccharide (C-O), and amid (C=O) groups found within the carbohydrate and protein constituents of probiotic cell walls [54]. Peptidoglycans contain disaccharides with pentapeptide bridges and may undergo modifications that include the addition of acetyl groups to both N-acetyl-glucosamine and N-acetyl-muramic acid. Numerous studies have indicated that the ability of PAT to bind to probiotics or potential probiotics varies depending on the cell wall structures. The involvement of S-layers directly in the bonds between probiotics and PAT has been reported [2]. The bacterial S-layers possess various attachment sites that are capable of adhering to the PAT via non-covalent bonds [53]. Also, probiotics have the ability to produce exopolysaccharides (EPS) from carbohydrates, which play a significant role in the biosorption process [60].

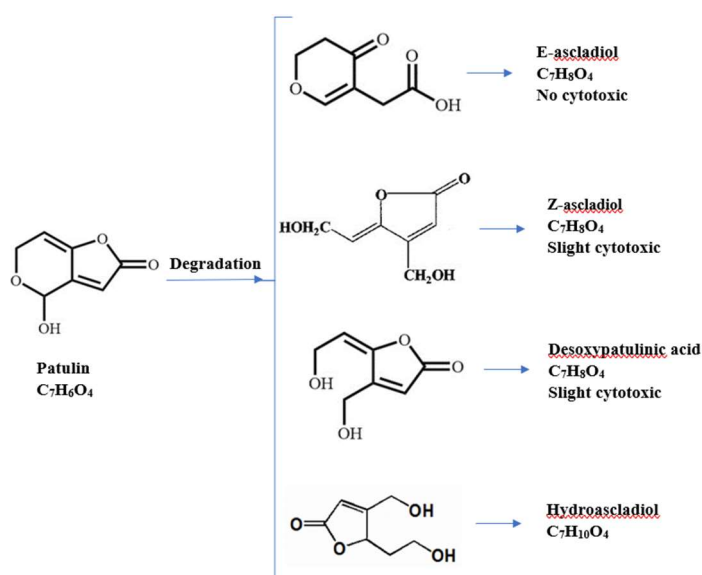
Wang et al. [49] discovered that LAB strains' efficiency in adsorbing PAT was due to the amino acid and starch components of their cell walls. Additionally, O, OH, and/or NH groups that are related to some protein and carbohydrate components were evidently involved in the adsorption process of PAT. Moreover, Zoghi et al. [6,53] emphasized the significance of S-layer proteins in reducing PAT in apple juice, which was supported by electrophoresis evaluation of *L. plantarum* and *L. acidophilus*. Both of these probiotics produced more S-layers at pH 3.5. Bahati et al. [45] have confirmed the involvement of alcohol phenol, O-H carboxylic acid, C=O amide I, and a C-O stretching bond in the adsorption of PAT.

### 9.2. Degradation

Some probiotics also can produce several proteolytic enzymes that help in PAT degradation and elimination [61]. PAT is degraded (**Figure 1**) to E-ascladiol and Z-ascladiol by *S. cerevisiae* via alcoholic fermentation [62], to hydroascladiol by *L. plantarum* [48], and to desoxypatulic acid by *Rhodospiridium paludigenum* [63]. The Z-isomer of ascladiol likely originates from E-ascladiol through the influence of cellular sulfhydryl compounds, including cysteine and glutathione. Degradation of



PAT to E-ascladiol can occur via an enzyme in the short-chain dehydrogenase family, reliant on NADPH (Nicotinamide Adenine Dinucleotide Phosphate). Presumably, the ascladiol produced can be further reduced to hydroascladiol by certain microorganisms [64]. The PAT degradation product from an orotate phosphoribosyltransferase enzyme has yet to be determined in apple juice but it has been hypothesized to be a phosphoribosyl modified PAT. Desoxyapatulinic acid is believed to be a metabolite of the hydrolysed 5-membered lactone ring of PAT [11]. The toxicology of PAT-related metabolites is less studied than that of PAT. Breakdown products of PAT with opened pyran rings demonstrated less cytotoxicity to human cell lines than PAT. However, no further reports have been made on their carcinogenicity, genotoxicity, or other toxic effects. PAT conjugates are formed due to its electrophilic attack on nucleophilic sulfhydryl groups, such as cysteine and methionine, in live cells. PAT can also target the cysteine-containing tripeptide glutathione (GSH). Although PAT/cysteine conjugates are deemed less or non-toxic to intestinal cell lines, the toxicological properties of 22 (or more) mass spectra identified PAT-GSH adducts remain unclear [48,62,65].



**Figure 1.** Degradation products of patulin.

### 9.3. Transformation

Some probiotics can produce metabolites like phenolic compounds, acids, fatty acids, and bioactive peptides that are associated with the removal process via interaction with PAT. PAT would react with the mentioned metabolites and change to some nontoxic forms. Also, PAT may react with the thiol group present in protein extracts of probiotics and therefore, the functional properties of PAT will be destroyed [61].

## 10. Conclusion

It is clear that PAT in fruits and derived products poses a significant risk to human health. There have been various physical and chemical methods devised to reduce PAT, but most are not easily accessible due to high cost and unsuitability for industrial

manufacturing or the risk of new chemical hazards. Generally, optimal detoxification methods ought to eliminate or remove the PAT, refrain from generating or leaving additional toxic substances, sustain nutritional value and product acceptability, and, if possible, avoid significant modification of product processing technology. Recently, more attention has been paid to biological methods, such as using probiotics and potential probiotics (both live and dead) for PAT reduction due to their high efficiency, environmental friendliness, ease, and safety of use. Adsorption by the probiotics cell wall, enzymatic degradation, and transformation of PAT to a less toxic compound are the mechanisms involved in the removal of PAT from fruit-based products. However, it remains unclear if there are any toxic effects caused by the by-products of PAT degradation, and the mechanisms underlying cellular PAT detoxification require further extensive investigation. Factors like the pH of the medium, PAT content, temperature, incubation time, probiotic strain and concentration affect the efficiency of PAT removal in fruit-based products using probiotics or potential probiotics. Therefore, further work is required to develop an optimal set of parameters to use in the fruit juice industry because most of the PAT bioremoval experiments were studied only on a laboratory scale. Additionally, there has been extensive research on reducing PAT in certain fruits and their by-products, specifically those from apple sources. However, there is a dearth of knowledge regarding bioremoval strategies for PAT in other fruits and their derived products. Generally, it is essential to develop effective control methods for PAT to guarantee the safety of fruits and their by-products in human diets.

**Conflict of interest:** The authors declare no conflict of interest.

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