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Phosphoribosyl Transferase (URA5) Enzyme-Mediated Detoxification of Patulin in Apple Juice and Its Effects on Nutritional Quality

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ABSTRACT: Patulin (PAT), a mycotoxin primarily produced by *Aspergillus* and *Penicillium* species, is a major contaminant in apple fruits and their derived products, posing significant health risks. The use of biocontrol approaches has gained attention due to their efficiency and minimal impact on product quality. This study investigated the enzymatic degradation of patulin by recombinant Orotate phosphoribosyltransferase and how it affects the nutritional properties of patulin-spiked apple juice. Apple juice samples were artificially spiked with patulin at concentrations of 100 µg/L and 250 µg/L and treated with the enzyme (0.1 mg/mL) at 36 °C for up to 24 hours. Post-treatment analysis included physicochemical properties such as pH, total soluble solids (TSS), acidity, viscosity, ascorbic acid content, total phenolic content, and amino acid composition. The enzyme-treated samples exhibited statistical reductions ($P<0.05$) in pH, TSS, ascorbic acid, and viscosity. Although decreases were observed in citric acid and total phenol content, these changes were not statistically significant. Among the amino acid fractions, isoleucine, leucine, arginine, proline, and serine remain unaffected ($P>0.05$), while a subset of essential and glucogenic amino acids showed statistically significant increases ($P<0.05$) in the treated sample. This study highlights the potential of enzymatic bioremediation as a safe and viable alternative for mitigating patulin contamination in fruit-based beverages. However, further studies are needed to confirm its efficacy and safety under industrial conditions.

Keywords: patulin, apple juice, orotate phosphoribosyltransferase, amino acids, nutrients

INTRODUCTION

Patulin (PAT) is a secondary metabolite produced primarily by *Penicillium expansum*, the causative agent of blue mold disease in stored apples (Sajid *et al*., 2019). It commonly contaminates apples and their derivatives—including juices, smoothies, and purees—and occasionally other fruits such as pears, peaches, bananas, and grapes (Li *et al*., 2019; Iqbal *et al*., 2018). Beyond the economic losses associated with fruit spoilage, the presence of patulin in food products poses serious health risks. It has been linked to a range of toxic effects in both humans and animals, including genotoxic, carcinogenic, immunotoxic, and neurotoxic outcomes (Donmez-Altuntas *et al*., 2013; Alam *et al*., 2014; Boussabbeh *et al*., 2016; Dural, 2020). Consequently, regulatory bodies such as the European Union and China have set a maximum permissible level of 50 µg/kg for patulin in apple juice and related products—standards now adopted by many countries, including South Africa (Rahimi & Jeiran, 2015; Tan *et al*., 2019).

Efforts to manage patulin contamination have included a variety of physical and chemical methods. However, patulin's high stability under acidic conditions makes it difficult to remove without compromising product quality (Sadok *et al*., 2019). Physical treatments are often labour-intensive and may alter the sensory and nutritional properties of fruit juices (Abbasi *et al*., 2019). Similarly, chemical approaches such as ozone and fungicide application can be effective but may introduce undesirable compounds or negatively affect the nutritional integrity of the product (Karaca & Velioglu, 2009; Cabanas *et al*., 2020). As consumer demand grows for minimally processed and functionally intact fruit products, it becomes essential to explore detoxification strategies that preserve the nutritional and physicochemical quality of juices.

Biological methods, particularly those involving microbial enzymes, are gaining traction due to their mild processing conditions and compatibility with food matrices. Among these, orotate phosphoribosyltransferase (OPRTase), a recombinant enzyme derived from *Cryptococcus neoformans var. neoformans* serotype D (URA5 strain AA 1-225), offers potential not only for mitigating toxin-related stress but also for supporting metabolic stability in fruit-based products. Unlike conventional detoxification agents, enzymes such as OPRTase may exert less disruptive effects on juice constituents, making them suitable for

maintaining key attributes like pH, vitamin content, total soluble solids, and antioxidant capacity.

This study, therefore, aimed to evaluate the influence of OPRTase treatment on the nutritional and physicochemical properties of apple juice artificially spiked with patulin. The enzyme, used in a purified form (≥90% purity), was selected for its availability, ease of application, and potential to interact safely with food-grade substrates. By assessing changes in juice quality parameters post-treatment, this study seeks to determine the suitability of OPRTase as a preservation aid that maintains the integrity of fruit juices even in the presence of mycotoxin contaminants.

MATERIALS AND METHODS

Materials and study area

The key raw materials used in this study included apple juice sourced from Granor Passi (Polokwane and Cape Town, South Africa), pure crystalline Patulin (PAT) standard obtained from Fermentek Biotechnology (Jerusalem, Israel), and Recombinant Cryptococcus neoformans var. neoformans serotype D Orotate phosphoribosyl transferase (URA5) (strain AA 1-225) (CSB-YP317769CTL biological reagent) with a purity of ≥ 90%, supplied by Inqaba Biotech Africa (Pty) Ltd (Pretoria, South Africa). Additionally, ethyl acetate, sodium carbonate, and chromatographic-grade acetonitrile acetic acid were procured from Biocom Biotech Africa (Pty) Ltd (Johannesburg, South Africa). The apple concentrates used were collected in Langkloof, located in the Western Cape region of Cape Town, where strict adherence to good manufacturing and agricultural practices was maintained throughout the apple-to-apple concentrate production process. A total of 8 samples of apple concentrate, with a concentration of 70 °Brix and a pH range of 3.2 to 4.10, were systematically collected at various processing stages. These samples were then aseptically stored at -16 °C to preserve their integrity for subsequent analysis. All experimental procedures and analytical work were conducted under laboratory-scale conditions at the University of Johannesburg.

Production of apple concentrate

Upon receiving the apples, they were checked for rottenness and the presence of fungal growth after washing with ozonated water. A quality check was then conducted on all batches to identify any rotten apples or visible fungal growth. Any batch where the proportion of internally rotten fruits exceeded the acceptable threshold for juice production was discarded (Figure 1). The washed apples were crushed, and the resulting mash was pumped into a mash tank before being transferred to belt press machines. The extracted juice obtained as the belts pressed against each other was collected into storage tanks. The fresh juice was then heated and cooled in preparation for pectinase enzyme treatment. Following enzymatic treatment, the juice underwent ultrafiltration to obtain a clear liquid. The filtered juice was pumped into a T.A.S.T.E. (thermally accelerated short-time evaporation) evaporator, where it reached a concentration of 70 °Brix.

Preparation of PAT stock, standard, and enzyme working solutions

A 25 mL volumetric flask containing 5 mg of pure crystalline patulin (PAT) was filled with 25 mL of ethyl acetate to create the stock solution, protected with aluminium foil, and stored in a freezer at -16 °C until further use. To calibrate the PAT standard stock concentration, 250 µL of the solution was evaporated to dryness under a nitrogen stream, then reconstituted in 1 mL of 100% ethanol (Lai et al., 2000). For PAT quantification, injections of 5, 10, 25, and 50 µg PAT/50 L were prepared, corresponding to concentrations of 0.1, 0.2, 0.5, and 1.0 µg PAT/mL in an acetic acid solution. This solution was prepared by adjusting the pH of distilled water to 4.0 using Glacial Extrapure AR (99.9%) acetic acid.

To prepare PAT working standards, 100 µL of the PAT stock solution was transferred into a 10 mL volumetric flask and evaporated to near dryness at room temperature under a nitrogen stream. The residue was immediately diluted with the acetic acid solution and thoroughly mixed. Aliquots of 50, 100, 250, and 500 µL were taken, and corresponding volumes of 0.95, 0.9, 0.75, and 0.5 mL acetic acid solution were added to 1 mL volumetric flasks. To create a series of standard working solutions, acetic acid was used to dilute PAT solutions to concentrations ranging from 100 to 500 µg/L, yielding final concentrations of 1, 2, 5, 10, 20, and 50 µg/L. Both original and diluted solutions were further used to prepare a range of PAT concentrations from 1 to 250 µg/L, which were then used to generate a calibration curve at 50, 100, 150, 200, and 250 µg/L.

OPRTase was provided in a lyophilized powder form. Before lyophilization, the buffer was a Tris/PBS-based solution containing 6% trehalose (pH of 8.0) as a stabilizing agent to protect the enzyme structure during

the lyophilization. The enzyme's purity (>90%), was verified using SDS-PAGE, and its molecular weight was confirmed after Immobilized Metal Affinity Chromatography (IMAC) (Abdelghafour, 2023). Peptide sequencing was conducted after cutting and purifying the SDS-PAGE gel (Chang et al., 2013).

Batch degradation experiments

A patulin (PAT) solution was used to spike two aliquots of apple juice, resulting in final concentrations of 100 µg/L and 250 µg/L, both exceeding the South African permissible limit of 50 µg/L for apple juices and apple juice-containing beverages. As a control group, unspiked apple juice samples were analyzed. Before testing, all solutions were frozen at -20 °C and then thawed overnight at 4 °C in the absence of light.

The PAT degradation assay was conducted in 1.5 mL Eppendorf tubes following the method described by Tang et al. (2019), with modifications. A 100 µL aliquot of orotate phosphoribosyl transferase (OPRTase) was mixed with 100 µL PAT-contaminated apple juice. The samples were placed in a shaker-incubator (Labcon 3081U, Labcon, South Africa) and incubated at 36 °C for 3, 6, 9, 12, 18, and 24 hours at 180 rpm.

After the 24-hour degradation period, samples were centrifuged at 8,000 rpm for 10 minutes for PAT extraction (Paterson et al., 2018). PAT was extracted using ethyl acetate, followed by a sodium carbonate solution for sample cleanup. The quality of the extracted samples was assessed by filtering the supernatant through a 0.22 µm syringe filter before LC-MS analysis to identify and quantify any remaining PAT. The degradation assay was performed in triplicate to ensure reliability and reproducibility. The results of the enzymatic degradation of patulin-spiked apple juice have been reported by Mapheto et al. (2025).

Physicochemical characterization of apple juice

In triplicate (n=3), the original apple products were compared to the treated apple samples for assessment in terms of pH, total soluble solids (TSS, °Brix), acidity (total titratable acidity), and ascorbic acid. All values were expressed as mean ± standard deviation.

pH

The pH of the treated apple juice samples was measured using a portable pH meter (Model HI8424, Hanna Instruments (Pty) Ltd., Africa), which was calibrated prior to use with standard buffer solutions of pH 4.00 and 7.00 (Hlangwanani, 2021).

Total soluble solids (TSS)

Total soluble solids (TSS) of the treated apple juice samples were determined using a digital refractometer (Model HI96801, South Africa) following the procedure described by Hlangwanani (2021). About 1 mL of each sample was placed into the refractometer well using a clean pipette. Measurements were taken in duplicate, and results were expressed in degrees Brix (°Brix), representing the proportion of soluble sugars in the sample.

Total titratable acidity (TTA)

Total titratable acidity (TTA) was measured using the method approved by AACC (2010) and described by Hlangwanani et al. (2024). In this procedure, 10 g of each treated sample was dissolved in 100 mL of distilled water and mixed thoroughly for approximately one minute. Next, 0.5 mL of 1% phenolphthalein was added as an indicator, and the solution was titrated with a standardized 0.1 N sodium hydroxide solution until a faint pink endpoint was observed. TTA was calculated and expressed as a percentage of lactic acid using the formula: %TTA = (mL NaOH × 0.009 × 100)/Volume of sample

Ascorbic acid

Vitamin C concentration in the treated apple juice samples was assessed following the procedure of Odriozola-Serrano et al. (2007), with slight modifications. In brief, 0.2 g of sample was extracted using 10 mL of 4.5% metaphosphoric acid and subjected to sonication in an ice-cooled ultrasonic bath for 30 minutes before filtration. Analysis was performed on a Shimadzu HPLC system (LC-2030C 3D, Shimadzu Corporation, Kyoto, Japan) fitted with a Luna C18 column (150 × 4.6 mm, 5 µm) maintained at 25 °C. The operating conditions included a 20 µL injection volume, a 245 nm detection wavelength, and an isocratic flow rate of 1 mL/min. The mobile phase consisted of water, acetonitrile, and formic acid in a 99:0.9:0.1 (v/v/v) ratio (Hao et al., 2016). Calibration was achieved using standard ascorbic acid solutions prepared in varying concentrations. Results for vitamin C were expressed as milligrams per 100 g dry weight (mg/100 g DW)

Determination of total phenolic content

Extraction of phenolic compounds followed the procedure outlined by Amoo et al. (2012). Approximately 0.2 g of treated apple juice sample was centrifuged at 1073.3 × g for 2 minutes, after which it was sonicated for 30 minutes in a cold-water ultrasonic bath containing 10

Table 1. PAT degradation in treated apple juice, recoveries in hrs, and mean values

Spiking level (µg/L)	Time (hrs)	Degraded Rate (%)	Final concentration (µg/L)	Recovered PAT (µg/L)/ or %	Mean concentration
100	3	18.18	84.36	15.61	84.36±0.01[a]
250		37.29	163.10	86.95; 34.80*	163.10±0.01[b]
100	6	57.27	57.30	42.71	57.27±0.05[a]
250		60.62	112.69	137.31; 54.93*	112.69±0.01[b]
100	9	64.55	52.25	47.75	52.25±0.01[a]
250		68.21	96.32	153.68; 61.47*	96.32±0.01[b]
100	12	67.81	49.99	50.01	49.99±0.00[a]
250		86.00	57.92	192.08; 76.833*	59.91±0.01[b]
100	18	71.82	47.22	52.78	42.22±0.00[a]
250		93.88	40.10	202.78; 81.11*	40.09±0.01[a]
100	24	96.36	30.22	69.78	30.22±0.01[a]
250		98.25	31.48	218.52 87.41	31.48±0.01[a]

* = % of recovered PAT at 250 µg/L, NB; µg/L = % at 100 µg/L PAT recovered and $P \leq 0.05$, means within column followed by the same letter do not differ significantly

mL of 50% methanol (Mabotja, 2020). The total phenolic content was quantified using a modified Folin–Ciocalteu assay described by Makkar (2003). Following the method of Wang et al. (2023), 50 µL of the sample was combined with 450 µL distilled water, 250 µL Folin–Ciocalteu reagent, and 1250 µL of 2% (w/v) sodium carbonate. The mixture was briefly vortexed and left to react at room temperature for 40 minutes. Absorbance readings were then taken at 725 nm using a Specord 210 Plus spectrophotometer (Analytik Jena, Jena, Germany). A gallic acid standard was used to generate a calibration curve (Thabit et al., 2018). All measurements were conducted in triplicate, and results were expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

Extractable amino acid compositions

Amino acid composition was determined following the method described by Adebiyi et al. (2017). Analyses were performed at the Irene Analytical Research Laboratory, Agricultural Research Council (ARC), Pretoria, South Africa, using high-performance liquid chromatography (HPLC).

For sample preparation, 700 mg of each treated apple juice sample was hydrolysed with 5 mL of 6 N hydrochloric acid in the presence of α-amino-β-guanidino propionic acid as the internal standard (Hlangwanani, 2021). After hydrolysis, the resulting mixtures were transferred to sterile Eppendorf tubes and centrifuged at 2000 × g for 10 minutes using an Eppendorf 5420 ce ntrifuge (Hamburg, Germany). The supernatants were filtered through 0.45 µm filters prior to analysis.

The obtained protein hydrolysates were evaporated to dryness under a nitrogen stream and reconstituted in borate buffer with the addition of the FMOC reagent (Sigma-Aldrich, Missouri, USA). The prepared solutions were analysed by HPLC equipped with a fluorescence detector (Perkin-Elmer LS-4 and Shimadzu RF-530) set at an excitation wavelength of 260 nm and an emission wavelength of 313 nm (Hlangwanani, 2021). The mobile phase composition was gradually shifted over 90 minutes from a methanol–acetic acid–acetonitrile mixture (50:40:10, v/v) to a 50:50 (v/v) acetonitrile–acetic acid mixture. Column temperature was maintained at 40 °C, with an initial flow rate of 1.3 mL/min for the first three minutes, followed by an increase to 2 mL/min for 30 seconds (Vierra, 2023). Quantification of amino acids was based on calibration curves prepared from standard amino acid solutions (Sigma-Aldrich, Missouri, USA).

Statistical analysis

Table 2: Nutrition analysis of treated apple juice products after degradation experiments

Product	TSS (g/100g)	AA (g/100g)	pH	MA (%)	CA (%)	Viscosity (cm/min)	Total phenol (mgGAE/g)
Standard	69.99±0.1[a]	13.43±0.0[a]	4.06±0.02[a]	1.48±0.01[b]	1.39±0.00	1266.7±104.1[a]	4.62±0.0
Treated sample	68.61±0.1[b]	12.09±0.0[b]	3.62±0.00[b]	1.51±0.01[a]	1.40±0.00	883.3±76.4[b]	4.22±0.0
P-Value	0.0027	0.0006	0.0008	0.0099	0.06	0.0000	0.06

TSS = Total soluble solids, AA= Ascorbic acid, MA= Malic acid, CA = Citric acid

Using IBM SPSS Statistics 22 (SPSS/IBM, Chicago, Illinois), analysis of variance (ANOVA) was utilized to compare the PAT concentration and PAT percentage degradation data (Bricker et al., 2014). The mean difference was performed using Turkey's tests and considered significant at $P<0.05$. All data were reported as mean±standard deviation.

RESULTS AND DISCUSSION

The impact of degradation on Total soluble solids (TSS), pH, and viscosity

After 24 hrs of degradation experiments (Table 1), the treated apple juice was analyzed to assess the nutritional composition (Table 2). Patulin that was recovered after 24 hrs was 69.78 ug/L at 100 ug/L spiking level and 87.41 ug/L at 250 ug/L spiking level with final concentrations of 30.22 and 31.42 ug/L, which are under the permissible limit of 50 ug/L Patulin in South Africa. The soluble solid content and pH decreased ($P<0.05$) from 69.99 to 68.61 and from 4.06 to 3.12 in the standard and treated apple juice, respectively. The observed decrease in the soluble content of the treated apple juice could be a result of a much-improved surface area of OPRTase on the sugars and its effective utilization (Zandi et al., 2016). The decreased pH is a reflection of the increase in the organic acid of the treated apple juice (Roberts et al., 2018).

The chemical stability of foods and beverages, including their microbiological, sensory evaluation, and nutritional properties, is greatly influenced by organic acids (Silva et al., 2018). Although the values obtained for the citric acid were similar ($P>0.05$), the treated apple juice had lower ($P<0.05$) ascorbic acid values compared to the standard untreated apple juice. However, the recorded value for malic acid was higher ($P<0.05$) in the treated than in the standard. In a similar study on the biodegradation of the mycotoxin patulin in apple juice by OPRTase from Rhodotorula mucilaginosa, Tang et al. (2019) reported a 4.51% and 6.55% increase in malic acid and citric acid, respectively, after degradation of patulin by OPRTase. Contributory factors to such an increase in organic acids have been attributed to various processes, including metabolic, biochemical, and hydrolysis (Flores et al., 2012; Escobar et al., 2013).

Ascorbic acid, though unstable under several processing conditions, is an important nutrient. It is an index that provides estimates of quality deterioration during processing (Serpen & Gokmen, 2007; Lima et al., 2010). In this study, the ascorbic acid was 13.43 in the standard and 12.09 ($P<0.05$) in the treated apple juice, losing about 9.97% to degradation activities. This loss was lower than the 20.843% reported by Tang et al. (2019). In a study conducted by Lu et al. (2018), the changes in ascorbic acid levels were attributed to variations in the pH, dissolved oxygen, and other metabolic processes.

Phenolic compounds, a major class of bioactive molecules, contribute significantly to the nutritional quality and health-promoting properties of foods (Al-Qassabi et al., 2018). In the present work, treatment with the enzyme did not produce a statistically significant change in patulin-contaminated apple juice. Nonetheless, the total phenolic content showed a slight decline, from 4.62 mg GAE/g in the control sample to 4.22 mg GAE/g in the enzyme-treated juice. Tang et al. (2019) reported a comparable reduction, which they associated with oxidative breakdown of reducing agents, including ascorbic acid and various phenolic constituents (Vinson et al., 2001).

Viscosity is a key fluid property in food systems, providing valuable insights into product quality assessment, process control, and the structural characterization of food materials (Krokida et al., 2001). As a branch of rheology, it describes how substances deform and flow under applied forces. In the current study, apple juice treated with the enzyme showed a significant reduction in viscosity ($P<0.05$), decreasing from 1266.7 cm/min in the control sample to 883.3 cm/min. The flow characteristics of fruit juices and their concentrates

Table 3. Amino acid profile of apple juice

Amino acid (g/100 g)	Standard original apple juice	Treated apple juice
Protein*	0.68	0.69
Essential		
Histidine	0.0045± 0.02[a]	0.005 ± 0.01[b]
Isoleucine	0.005 ± 0.00	0.005 ± 0.01
Leucine	0.002 ± 0. 29	0.003 ± 0.29
Lysine	0.003 ± 0.02[a]	0.0035 ± 0.01[b]
Methionine	0.005 ± 0.05[a]	0.003 ± 0.00[b]
Phenylalanine	0.004 ± 0.01[a]	0.002 ± 0.00[b]
Threonine	0.006 ± 0.05[a]	0.008 ± 0.05[b]
Valine	0.005 ± 0.025[a]	0.006 ± 0.01[b]
Nonessential		
Alanine	0.010 ± 0.01[a]	0.012 ± 0.0 [b]
Arginine	0.003 ± 0.0	0.003 ± 0.01
Aspartic acid	0.361 ± 0.0 [a]	0.475 ± 0.0 [b]
Glutamic acid	0.055 ± 0.0 [a]	0.061 ± 0.01[b]
Glycine	0.003 ± 0.0 [a]	0.004 ± 0.00[b]
HO-Proline	0.002 ± 0.0	0.002 ± 0.01
Serine	0.017 ± 0.01	0.018 ± 0.22
Tyrosine	ND	ND
Total AA	0.4855	0.6105

*For the conversion of nitrogen to protein the factor 6.25 was used; g = gram; HO – hydroxy; ND – not detected. Within a row, mean values with distinct superscripts demonstrate a substantial difference at ($p < 0.05$).

largely depend on their composition, particularly the fruit type, and the processing techniques applied. Fruit juices with low °Brix values, especially when processed under high temperatures, may behave as Newtonian fluids, whereas many concentrates and juices, such as those from orange, pomelo, and grape, display non-Newtonian, shear-thinning behavior (Salehi, 2020). Viscosity tends to increase with higher total soluble solids (TSS) and decrease as temperature rises (Salehi, 2020). The notable decline in TSS observed in the treated sample accounts for the corresponding drop in its viscosity.

Degradation's effects on amino acid profile

The amino acid profile of treated and untreated apple juice is shown in Table 3. Despite being present in small amounts in juices, amino acids are important juice quality parameters (Odriozola-Serrano et al., 2013). The addition of the enzyme to the patulin-spiked apple juice had no effect ($P>0.05$) on isoleucine, leucine, arginine, proline, and serine. However, the treated apple juice had a much higher ($P<0.05$) histidine, lysine, threonine, valine, alanine, aspartic acid, glutamic acid, and glycine. Only methionine declined in concentration in the treated apple juice. Tang et al. (2019) also reported a decline in methionine, as well as aspartic acid and cystine concentrations in the enzyme-treated patulin-spiked apple juice. Being an enzyme that can easily be decomposed to constituent amino acids, the increase in some amino acids in this study could be attributed to the degradation of the enzyme Orotate phosphoribosyl transferase in the treated apple juice. Another explanation could be due to the relationship between pH and amino acid loss. According to Labuza and Baisier (1992), a pH range of 2-10 could

lead to a loss of amino acid. Hence, suffice it to say that the decrease in pH in the treated apple juice in this study shielded the amino acids from loss.

CONCLUSION

This study evaluated the degradation effect of OPRTase on the nutritional properties of patulin-spike in apple juice. While minor alterations in physicochemical properties were observed, such as reductions in pH, total soluble solids, viscosity, and ascorbic acid content, these changes did not compromise the overall nutritional value of the juice. The enzyme treatment also influenced the amino acid profile, with an increase in specific essential and non-essential amino acids, possibly due to enzymatic degradation. These findings suggest that OPRTase presents a promising alternative to conventional patulin detoxification methods, which often have drawbacks such as nutrient loss, chemical residues, and high processing costs. However, further studies should explore the enzyme's effectiveness in different fruit matrices, optimize treatment conditions for large-scale applications, and assess the long-term storage stability of treated products.

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