Research Note

Ability of Inactivated Yeast Powder To Adsorb Patulin from Apple Juice

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ABSTRACT

This study aimed to investigate the adsorption of patulin from apple juice, using two types of inactivated yeast powder: laboratory-prepared yeast powder (LYP) and commercial yeast powder (CYP). The effects of incubation time, pH, incubation temperature, adsorbent amount, and initial concentration of patulin and the stability of the yeast-mycotoxin complex were assessed. The results showed that the efficiencies of the two yeast types in adsorbing patulin were similar. The ability of the powders to remove patulin increased with longer incubation times, and patulin concentration was below detectable levels with LYP and CYP at approximately 36 and 30 h, respectively. The highest removal of patulin was achieved at pH 5.0 for both powder types, and there were no significant differences in patulin decrease at different temperatures (4, 29, and 37°C). Additionally, the adsorption percentage of patulin increased significantly with the increase of absorbent amount and decrease of initial concentration of patulin. Stability of the yeast-patulin complex was assessed, and patulin was more stable when washed in phosphate-buffered saline (pH 4.0) than in absolute ethyl alcohol. These results suggest that inactivated yeast powder has potential as a novel and promising adsorbent to bind patulin effectively.

Patulin [4-hydroxy-4H-furo(3,2-c)pyran,2(6H)-one] is a secondary metabolite produced mainly by certain species of Penicillium, Aspergillus, and Byssochlamys (5, 13, 30). Among these, Penicillium expansum is the most commonly encountered species (2, 9). This mycotoxin can be found in a number of plant products, including apples, pears, grapes, apricots, strawberries, blueberries, and peaches (22), and frequently occurs in commercial fruit juices, especially apple juice (9, 21). High levels of patulin, up to 2,500 μg/kg, have been found in apple juice obtained from rotten apples (9). Apple juice contaminated with patulin continues to be a problem for human health, due to its toxicity. It has been reported that patulin has carcinogenic, mutagenic (21), immunotoxic, genotoxic, and neurotoxic (18) effects against animals. It also causes liver, spleen, and kidney damage and weakness of the immune system (19). Therefore, in 1995, the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives discussed the provision of a maximum tolerable daily intake of 0.4 μg/kg of body weight. Additionally, a maximum recommended concentration of 50 μg of patulin per liter in apple juice was established by the World Health Organization (28).

In order to eliminate this mycotoxin, several physical, chemical, and biological methods have been shown to reduce patulin contamination in apple juice. Treatments such as washing, clarification, and filtration in processing have been tested and eliminate 54% of the initial patulin (1). It was reported that gamma irradiation (30) or UV radiation (7) could reduce patulin concentration in apple juice or cider. Although these methods could maintain basic sensory and quality attributes, the processes have not been found to be successful. Certain chemical methods have been reported to reduce patulin concentration in apple juice by changing patulin into less toxic products. These include the use of ammoniation and potassium permanganate oxidation (14) or organic acids and vitamins (4, 29). However, such treatments introduce additional chemical hazards during processing and in certain cases are not allowed for commodities destined for human consumption (16). This has led to new opportunities for microbiological removal of patulin. Previous research indicated that some microorganisms, especially bacteria, have a protective effect against mycotoxin contamination (15, 24, 27). In recent years, yeasts have also been reported to have high adsorption of mycotoxins. Coelho et al. (5) indicated that Saccharomyces cerevisiae was able to remove more than 90% patulin after incubation at 25°C under static conditions. Moreover, the patulin level was reduced by over 99% during alcoholic fermentation of apple juice to wine by S. cerevisiae (26). In 2004, Bejaoui et al. (3) reported that dead yeast cells could be used for removing ochratoxin A from grape juice without posing any quality or safety problems.

In the present study, we investigated the potential for patulin removal from contaminated apple juice (12.5°Brix) by inactivated yeast powder. The objectives of this study were to (i) investigate the potential for patulin removal from...
apple juice by inactivated laboratory-prepared yeast powder (LYP) or by commercial yeast powder (CYP); (ii) study the influence of the patulin removal by inactivated yeast powder in different adsorptive conditions (incubation time, pH, incubation temperature, adsorbent amount, and initial concentration of the toxin); and (iii) examine the stability of the yeast-mycotoxin complexes formed, so that the mechanism of toxin removal might be better understood.

**MATERIALS AND METHODS**

**Materials.** Standard patulin and acetonitrile (high-performance liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich (St. Louis, MO). Phosphate-buffered saline (PBS, pH 4.0) was adjusted with 0.1 M HCl. All other chemicals used were of analytical reagent grade and were purchased from a local manufacturer, Chemical Reagent Company (Yangling, China).

The *S. cerevisiae* strain (coded YS3) that was used for laboratory preparation of yeast powder was obtained from the Fermentation Laboratory at the College of Food Science and Engineering of Northwest Agriculture and Forestry University (Yangling, China). Commercial active dry yeast powder of *S. cerevisiae* (Enoferm Assmannshausen) was purchased from Scott Laboratories (Petaluma, CA).

**Yeast preparation.** Strain YS3 was cultivated in liquid yeast extract peptone dextrose medium (2% yeast extract, 2% peptone, and 2% glucose) for 24 h at 30°C. After incubation, yeast cells (10^10 CFU/ml) were collected by centrifugation (PM180R, SIM International Group Co. Ltd., Newark, DE) at 3,600 × g for 20 min. The cultured biomass was washed twice with sterilized water and freeze-dried at −54°C for 26 h by a vacuum freeze-dryer (MCFD5505, SIM International Group Co. Ltd.) to obtain activated LYP. To obtain inactivated cells, LYP and CYP were autoclaved for 20 min at 121°C and then used in subsequent adsorption experiments.

Cell viability was confirmed by methylene blue staining. A drop of methylene blue (1%, wt/vol) was mixed with a drop of cells onto a microscope slide and viewed by light microscopy after 10 and 30 min.

**Patulin binding assay.** Samples for all experiments were prepared by using a standard stock solution of patulin dissolved in ethyl acetate with the concentration of 200 µg/liter. The organic solvent was evaporated by heating in a water bath (40°C, 15 min), and the patulin was redissolved in the sterile apple juice (pH 4.0, 12.5° Brix, with no patulin detected by HPLC) diluted from apple juice concentrate (Hengxing Co., Baoji, China) to obtain the test solution.

Inactivated yeast powder (1.0 g) was added into a 150-ml sterile conical flask containing 40 ml of patulin test solution. The control was prepared without the addition of adsorbents. The control and test solutions were placed on a shaker-incubator (CertomatWR, B. Braun, Melsungen, Germany) at 120 rpm and 29°C for 24 h. The effects of different conditions (incubation time, pH, incubation temperature, adsorbent amount, and initial concentration of patulin) were assayed (Table 1). After the incubation period, the biomass was centrifuged and collected for release experiments. The supernatants of each strain were collected for the detection of patulin levels by HPLC.

**Patulin release assay.** Yeast pellets bound with patulin were suspended in 4 ml of PBS (pH 4.0) or absolute ethyl alcohol and incubated for different times (30 and 60 min) at 29°C with shaking. Subsequently, the yeast cells were centrifuged (3,600 × g, 20 min) and the supernatant was collected for quantification of released patulin.

**Sample extraction and patulin detection and quantification.** Samples were extracted and analyzed by a modification of the AOAC official method 2000.02 for the detection and quantification of patulin in clear and cloudy apple juices and apple puree (20). In this method, patulin was extracted three times with 20 ml of ethyl acetate and cleaned up by extraction with 4 ml of a 1.5% (wt/vol) sodium bicarbonate solution. This cleaned organic phase was passed over a bed of anhydrous sodium sulfate (15 g) and evaporated to dryness. Patulin was redissolved in 1 ml of deionized water, adjusted to pH 4.0 with acetic acid. HPLC analysis was performed immediately after all samples were filtered through a 0.22-µm-pore-size membrane.

Samples were analyzed with an HPLC system (Shimadzu LC-20AD pump, CTO-20A column oven, and SPD-M20A UV/Vis detector) and an Alltima reversed-phase column C18, inner diameter, 250 by 4.6 mm; 5-µm particles). A 20-µl sample was injected, and deionized water-acetonitrile (10:90, vol/vol) was used as isocratic mobile phase with a flow rate of 1 ml/min at 30°C. The detection wavelength was set at 276 nm. The percentage of patulin bound to the yeasts was calculated by the following equation.

\[
\text{% Removal} = 100 \times \left[ 1 - \left( \frac{\text{Peak area of patulin supernatant}}{\text{Peak area of patulin in the positive control}} \right) \right]
\]

**Statistical analysis.** All experiments were performed in triplicate, and the data were presented as means ± standard deviations (SD). Data were subjected to one-way analysis of variance by using the Statistical Analysis System (SAS version 9.1,
RESULTS

Effect of incubation time on patulin adsorption. The results clearly showed that inactivated yeast removed patulin. Removal increased with longer incubation times, and LYP had a higher adsorption percentage of patulin within 0 to 6 h (Table 2). The removal percentages were 12.33% for LYP and 10.30% for CYP at 0 h, indicating that the main mechanism is probably a rapid attachment of molecules to the surface of the yeast. Thereafter, patulin concentration was below the detection level (4.6 μg/liter) at approximately 36 and 30 h for LYP and CYP, respectively.

Effect of pH on patulin adsorption. As shown in Figure 1A, pH significantly influenced patulin adsorption by inactivated LYP and CYP. The patulin assay was conducted at pH 3.0 to 5.0 in apple juice; patulin has a high stability in the range of pH 2.5 to 5.5 (8). The results showed that the removal of patulin was highest at pH 5.0 for both types of inactivated yeast powder, and the rates of removal of patulin by LYP and CYP were 73.66 and 83.12%, respectively, at pH 5.0.

Effect of temperature. The effects of temperature on the adsorption behavior of patulin at different times were studied. Three temperatures were considered: 4, 29, and 37°C. Figure 2B showed that there was no significant difference (P > 0.05) on patulin adsorption by the two yeast types at the different temperatures investigated. This indicates that temperature has little influence on patulin adsorption.

Effect of adsorbent amount. It can be seen in Figure 2A that adsorption percentage of patulin by inactivated LYP and CYP depends strongly on the amount of adsorbents, as adsorption increased with adsorbent concentration. The results showed that the adsorption percentages of patulin by the two yeast types increased from 23.95 to 100% and from 20.46 to 100% for LYP and CYP, respectively, with increasing adsorbent amount from 0.1 to 3.0 g/40 ml.

SAS Institute Inc., Cary, NC. Statistical significance was considered to exist when P values were <0.05.

![Image: Figure 1A showing effect of pH on patulin adsorption by inactivated laboratory-prepared yeast powder and commercial yeast powder (1.0 g/40 ml) from apple juice at 29°C; Figure 1B showing pH values in apple juice after 0 to 48 h of incubation.]

![Image: Table 2: Adsorption of patulin from apple juice by inactivated yeast powder at 29°C after 0 to 48 h of incubation.]

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>Laboratory-prepared yeast powder</th>
<th>Commercial yeast powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.33 ± 0.07</td>
<td>10.30 ± 0.05</td>
</tr>
<tr>
<td>6</td>
<td>37.09 ± 0.14</td>
<td>35.92 ± 0.37</td>
</tr>
<tr>
<td>12</td>
<td>46.64 ± 0.17</td>
<td>54.74 ± 0.04</td>
</tr>
<tr>
<td>18</td>
<td>53.93 ± 0.05</td>
<td>64.83 ± 0.16</td>
</tr>
<tr>
<td>24</td>
<td>70.02 ± 0.46</td>
<td>75.73 ± 0.22</td>
</tr>
<tr>
<td>30</td>
<td>88.91 ± 0.18</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>36</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>48</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
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*Values are averages ± SD for duplicate samples.*
Effect of initial concentration of patulin. The effects of initial concentration of patulin on toxin adsorption by inactivated LYP and CYP are shown in Figure 3. Patulin adsorption was dependent on toxin concentration for both yeast powders, and the percentage removed was significantly influenced by concentration of patulin. The amount of toxin adsorbed increased with increasing patulin concentration; however, the overall adsorption percentage decreased with increasing toxin concentration.

Release study. The amounts of patulin released from inactivated LYP and CYP after washing with PBS (pH 4.0) and absolute ethyl alcohol at 30 and 60 min were determined (Table 3). The results indicated that patulin was not tightly adsorbed to either of the yeast powders. There was no significant difference between LYP and CYP as to patulin release percentage. However, the yeast-patulin complex was significantly more stable after washing with PBS (pH 4.0) than with absolute ethyl alcohol. The percentages of release of patulin for LYP and CYP, respectively, by PBS (pH 4.0) were 15.46 and 17.52% at 30 min and 21.59 and 21.77% at 60 min; the percentages by absolute ethyl alcohol were 30.65 and 29.23% at 30 min and 35.22 and 36.19% at 60 min.

DISCUSSION

The presence of patulin in commercial apple juices throughout the world indicates that, to a certain extent, the mycotoxin is stable throughout the steps used in the apple juice manufacturing process. In the present study, it was shown that inactivated yeast powder is able to remove patulin from apple juice. Therefore, this method could be applied in the processing of apple juice or apple juice concentrate. Furthermore, time, pH, temperature, adsorbent amount, and initial patulin concentration influence the patulin removal by inactivated yeast powder.

The removal of patulin by inactivated yeast powder increased with time. This is in agreement with Topcu et al. (27), who reported that detoxification of patulin by nonviable cells of Enterococcus faecium M74 and EF031.
increased with time. The removal rate of patulin was 15.8 to 21% at 1 h and increased to 41.6 to 45.3% after 48 h. In addition, Peltonen et al. (24) indicated that the removal of aflatoxin B₁ by *Lactobacillus amylovorans* CSCC 5160 increased from 52.6% at 0 h to 73.2% at 72 h. Meanwhile, the pH of the apple juice increased slowly with incubation time, but there was no significant difference between original apple juice and treatments after 12 h of incubation. This indicated that shorter incubation times contributed to stability of treated product. Therefore, it is possible that a combination of stirring for 12 h (patulin adsorption values of 46.6% and 54.7% for LYP and CYP, respectively) with inactivated yeast powder and filtering might reduce the levels of patulin in the processing of apple juice concentrate.

The maximum removal of patulin by yeast powder was observed at pH 5.0 for both powder types. Several researchers have also investigated the effect of pH on biosorption of mycotoxins by using different microbial biomasses and found similar results. Ringot et al. (25) found that optimal removal of patulin and ochratoxin A by lactic acid bacteria was at pH 5.0. Similarly, Zinedine et al. (31) showed that more aflatoxin B₁ removal takes place at pH 5.5 than at pH 3 or pH 4.5. However, Topcu et al. (27) noted that the highest detoxification of patulin by nonviable cells of *Enterococcus* strains occurred at pH below 5; this may be due to the effect of hydrogen bond interactions on removal of patulin.

The temperatures evaluated (4, 29, and 37 °C) did not affect the adsorption of patulin by either yeast powder, indicating that the binding process is energy independent. These results are in contrast to the findings of Zinedine et al. (31), who noted that optimal removal of aflatoxin B₁ by lactic acid bacteria was achieved at 25 °C. Toxins may have been metabolically converted via reactions with specific enzymes that are sensitive to incubation temperature.

Our results demonstrated that the amount of adsorbent was a major factor in patulin adsorption. Similar findings were made in earlier reports of zearealenone binding (11, 13). Additionally, it was observed that adsorption of aflatoxin B₁ by lactic acid bacteria and *S. cerevisiae* was adsorbent amount dependent (6). Moreover, the data in our study showed that adsorption of patulin was toxin concentration dependent and that the amount of toxin absorbed increased with increasing patulin concentration. Similarly, El-Nezami et al. (10) reported that the amount of aflatoxin B₁ removed by selected dairy strains of lactic acid bacteria increased with increasing concentration of aflatoxin B₁. Furthermore, Mateo et al. (23) indicated that the percentage of ochratoxin A reduction was higher in 2 μg/liter ochratoxin A culture (30%) than in 5 μg/liter culture (23%), showing that reduction was dependent on the initial toxin concentration.

It was observed that variable amounts of patulin were released from the yeast-patulin complexes after washing by pH 4.0 PBS or absolute ethyl alcohol. The results indicate that the adsorption of patulin was partially reversible. Similar findings were reported by Haskard et al. (17), who noted that binding of aflatoxin B₁ by lactic acid bacteria was reversible after five washes. In addition, El-Nezami et al. (12) demonstrated that aflatoxin B₁ binding by *Lactobacillus rhamnosus* GG was partially reversible. In this work, the findings confirmed the mechanism of patulin adsorption, whereby the patulin is attached to the yeast by weak, noncovalent interactions that could be at least partially reversible and cross-linked adsorption prevents the liberation of patulin.

As a conclusion, both LYP and CYP significantly reduced the levels of patulin. In addition, biosorption of patulin using inactivated yeast powder is an effective and profitable choice of biological control to avoid contaminated apple juice without risk of fermentation. Further studies are needed on the mechanism of patulin adsorption with different components of yeast cell wall.

**ACKNOWLEDGMENTS**

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**REFERENCES**


