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RESEARCH ARTICLE

Recombinant Laccase-Mediated Detoxification of Aflatoxins and Ochratoxin A in Meat Products: Occurrence, Correlation, and Storage Dynamics

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The current study examined the fungal contamination and associated mycotoxins, including aflatoxins (AFLs) and ochratoxin A (OTA), in selected beef products and evaluated the detoxifying efficacy of recombinant laccase. A total of 150 samples were evaluated, including burgers, sausages, minced meat, luncheon, and hawawshi (30 of each). Yeast and mold contamination was detected in 36.0% of samples, with values ranging from 2.24 to 4.35 log10 CFU/g. The incidence of AFLs and OTA was 30.7% and 22.7%, respectively. The highest levels of contamination occurred in sausages. The average AFLs and OTA residues were 5.76–16.38ppb and 0.86–3.72ppb, respectively. A positive association between fungal numbers and mycotoxin levels was found ($p<0.05$). The use of recombinant laccase in the treatment caused a considerable reduction of both toxins in a dose- and time-dependent manner during refrigerated storage, with the maximum reduction at 4% enzyme concentration. AFLs were more susceptible to deterioration than OTA. These results indicate the presence of mycotoxins in beef products and suggest the possible use of recombinant laccase as a sustainable approach for mycotoxin decontamination in meat systems.

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INTRODUCTION

Mycotoxins are poisonous secondary metabolites produced by filamentous fungi such as Aspergillus, Penicillium, and Fusarium, and pose a serious global threat for food safety due to their carcinogenic, hepatotoxic, and immunosuppressive effects (Ulusoy *et al*., 2022). The most often identified and dangerous mycotoxins among them are aflatoxins (AFLs) and ochratoxin A (OTA), which are often found in many food commodities, including animal-derived products (Nady *et al*., 2026). Traditionally, mycotoxins are associated with plant-based foods. However, there is increasing evidence of their prevalence in meat and meat products, mainly due to carry-over from contaminated feed and post-processing contamination (Pandey *et al*., 2023). Moreover, in developing nations, climatic factors, inadequate storage, and poor hygiene standards further increase the risk of mycotoxin contamination in meat products, creating major public health problems (El-Khodary, 2025).

Mycotoxins are very difficult to control in food systems due to their chemical stability and resistance to traditional processing methods such as heating and cooking (Syraji *et al*., 2025). Traditional detoxification procedures, whether physical or chemical, frequently have drawbacks, such as partial elimination, nutritional losses, and safety concerns. Biological detoxification methods are therefore of growing interest, especially the use of enzymes, due to their specificity, environmental safety, and minimal impact on food quality (Aasa *et al*., 2026). Among these, laccase enzymes have attracted great attention due to their ability to oxidize a variety of hazardous chemicals, including aflatoxins and OTA, into less harmful metabolites (Kameshwar *et al*., 2018; Jia *et al*., 2024). However, the application of recombinant laccase in real food matrices, especially meat systems, is still little investigated despite promising laboratory results.

Several studies have examined the enzymatic degradation of mycotoxins in cereals, dairy products, and liquid food systems. Still, little information is available on the application of recombinant enzymes for mycotoxin detoxification in meat products, which are complex food matrices rich in proteins and lipids. In particular, evidence regarding the simultaneous reduction of AFLs and OTA in processed meat products under refrigerated storage conditions is sparse. Moreover, the association of fungal

infection with mycotoxin presence in retail meat products has not been adequately correlated with enzymatic detoxification techniques.

Based on this background, the hypothesis was that greater fungal contamination is associated with increased mycotoxin levels and that recombinant laccase can effectively reduce aflatoxins, AFLs, and OTA in meat products in a dose- and time-dependent manner. This study combines yeast and mold contamination assessment, detection of AFLs and OTA occurrence and residues, their correlation evaluation, and deployment of a recombinant laccase system for detoxification in a real beef matrix during storage. The purpose of this study was therefore to explore the presence of fungal infection and associated mycotoxins in some selected beef products and to assess the efficacy of recombinant laccase enzyme in the degradation of aflatoxins and ochratoxin A during refrigerated storage.

MATERIALS AND METHODS

Sample Collection: A total of 150 beef product samples, including burgers, sausages, minced meat, luncheon, and hawawshi (30 samples each), were randomly collected from various supermarkets from May to August 2024. Each sample (approximately 100 g) was aseptically collected, placed in sterile polyethylene bags, and transported to the laboratory in insulated ice boxes. Upon arrival, samples were kept in their original packaging and stored at 4°C until further analysis.

Chemicals and materials: Commercial laccase (from *Trametes versicolor*, Sigma-Aldrich, Germany) was used. Aflatoxins (34031-2ML-R) and ochratoxin A (34037-2ML-R) standards (purity > 99%) was obtained from (Sigma-Aldrich, Germany). 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was purchased from (Sigma-Aldrich, Germany). Chemicals including boric acid, acetic acid, phosphoric acid, citric acid, sodium dihydrogen phosphate and sodium hydroxide all were of analytical grade purchased from (Sigma-Aldrich, Germany). Britton–Robinson (BR) buffer solutions (pH 2–10) were prepared by mixing equal volumes (10mL each) of 0.04 mol L^{-1} boric acid, acetic acid, and phosphoric acid in a 100mL volumetric flask containing approximately 50mL of double-distilled water. The pH was adjusted to the desired value by the dropwise addition of 0.2mol.L^{-1} sodium hydroxide (NaOH) solution. The final volume was then completed to 100mL using double-distilled water. Deionized water obtained from a Milli-Q ultrapure water purification system was used throughout all experiments.

Assessment of yeast and mold contamination

Sample Preparation: Ten g of each sample were aseptically weighed and homogenized with 90mL of sterile buffered peptone water (M614, HiMedia) to obtain a 10^{-1} dilution. Serial decimal dilutions were prepared up to 10^{-5} as required (El-Hawary *et al*., 2025).

Enumeration of yeasts and molds: Yeast and mold counts were determined using the pour plate technique on Potato Dextrose Agar (MH096, HiMedia) supplemented with chloramphenicol to inhibit bacterial growth. Inoculated 1mL of appropriate dilutions was plated in duplicate and incubated for 5 days at 25°C (Nady *et al*., 2023; Zaki *et al*., 2023). After incubation, colonies were counted and expressed as colony forming units per gram (CFU/g)

Determination of Aflatoxins and Ochratoxin A: Quantitative analysis of AFLs and OTA was performed using competitive direct enzyme-linked immunosorbent assay (CD-ELISA), following the method described by (Elbarbary *et al*., 2025).

Commercial ELISA kits (RIDASCREEN® Aflatoxin Total, Art. No. R4701, and RIDASCREEN® Ochratoxin A 30/15, Art. No. R1312; R-Biopharm AG, Darmstadt, Germany) were used according to the manufacturer's instructions. Methods of validation including LOD and LOQ, Recovery (%), Precision, and calibration curve were mentioned in the Supplementary File.

Preparation and production of laccase enzyme: Recombinant laccase enzyme was expressed as a heterologous expression host in *Saccharomyces cerevisiae*. The gene encoding laccase was isolated from a fungal source and subcloned into a yeast expression vector under a galactose-inducible promoter to optimize extracellular enzyme production. Transformed *S. cerevisiae* cells were grown in S-Gal media with galactose as the inducer and trace levels of copper sulfate ($CuSO_4$), a cofactor necessary for laccase activity. The cultivation was carried out at 30°C for 4 days with constant agitation (160 rpm) in a rotary shaker incubator to ensure sufficient aeration and optimal enzyme secretion.

After incubation, the culture was centrifuged at 9,190 × g for 30 min to pellet the yeast biomass. The supernatant containing the extracellular crude recombinant laccase was carefully collected and used for further processing (Zaccaria *et al*., 2023).

Purification of laccase: The crude enzyme extract was partially purified according to the method reported by Liu *et al*. (2014). In brief, proteins were precipitated with ammonium sulfate saturation (60–80%) and recovered using centrifugation to obtain the enzyme fraction. The precipitate was then redissolved in sodium acetate buffer (pH 5.0) and dialysed against the same buffer to remove residual salts. Laccase, partially purified, was kept at 4°C until usage. A purification summary table has been added to the Supplementary File (Table S1).

Determination of laccase activity: Laccase activity was evaluated spectrophotometrically using ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as the substrate, as described by Liu *et al*. (2014) with minor modifications. The reaction mixture comprised 2.7mL of sodium acetate buffer (0.1 M, pH 5.0), 0.2mL of ABTS solution (1 mM), and 0.1mL of enzyme extract, for a final reaction volume of 3.0mL. The reaction was incubated at 30°C, and the increase of absorbance owing to ABTS oxidation was monitored at 420nm for 3min by a spectrophotometer (Shimadzu UV-1800, Japan).

The laccase activity was assayed by the molar extinction coefficient of oxidized ABTS. One unit (U) of laccase activity was defined as the amount of enzyme

required to oxidize 1 µmol of ABTS per min under the test conditions.

Application of laccase in mycotoxin detoxification: Burger samples were prepared according to the procedure of Elbarbary *et al*. (2024) with slight modifications. In summary, ground beef and fat were blended in a Seydelmann spiral mixer (Urgstallstraße, Germany) to form a homogeneous meat mass. Then, the mixture was spiked with AFLs and OTA standards at 100 µL/L to imitate contaminated circumstances.

The contaminated meat batter was treated with varying quantities (1-4%, v/w) of the laccase enzyme (partially purified), and the samples were separated into five experimental groups: C (control, without laccase), L1 (1%), L2 (2%), L3 (3%), and L4 (4%). Each batch was mixed with a stomacher for 5 min to allow a homogeneous distribution of both mycotoxins and the enzyme (Elbarbary *et al*., 2025).

Storage conditions and sampling intervals: The treated samples were aseptically packed into sterile polyethylene bags and stored at 4°C under refrigeration. Samples were tested at specific time points (0, 3, 5, 7, 9, 11, 13, and 15 days) to track temporal variations (Dandrawy *et al*., 2026).

At each sampling point, the aforementioned CD-ELISA method was used to quantify AFLs and OTA levels to evaluate laccase detoxification efficiency during storage.

Statistical Analysis: All experiments were performed in triplicate, and data were expressed as mean ± standard error (SE). Statistical analysis was carried out using SPSS software (version 16.0; IBM Corp, USA). One-way or two-way analysis of variance (ANOVA) was used to determine the significance of differences among means, as appropriate. For post hoc comparisons, Duncan's multiple-range test was used. The association between categorical variables was evaluated using the Chi-square (χ^2) test, and Pearson's correlation coefficient (r) was used to determine the association between yeast and mold counts and mycotoxin levels. Differences were judged statistically significant at $p<0.05$.

RESULTS

Yeast and mold counts: Yeast and mold contamination was detected in 36.0% (54/150) of the examined beef product samples, with variable prevalence among product types (Table 1). The highest proportion of positive samples was observed in sausage (50.0%), followed by burger (43.3%), hawawshi (36.7%), and luncheon (30.0%), while minced meat exhibited the lowest prevalence (20.0%).

Quantitatively, yeast and mold counts showed significant differences among the examined products ($p < 0.05$). The highest mean count was recorded in sausage samples (4.35 ± 0.02 $\log_{10}$ CFU/g), followed by burger (3.89 ± 0.03 $\log_{10}$ CFU/g) and hawawshi (3.45 ± 0.06 $\log_{10}$ CFU/g), with no significant difference between the latter two groups. Lower counts were observed in luncheon (2.93 ± 0.01 $\log_{10}$CFU/g), while minced meat samples exhibited the lowest level of contamination (2.24 ± 0.05 $\log_{10}$ CFU/g).

However, the differences in the occurrence of yeast and mold among the examined beef products were not statistically significant ($\chi2=6.12$, $p=0.19$) by chi-square ($\chi2$) analysis. This indicates that fungal contamination was observed across all product categories, but no significant variation in frequency was observed.

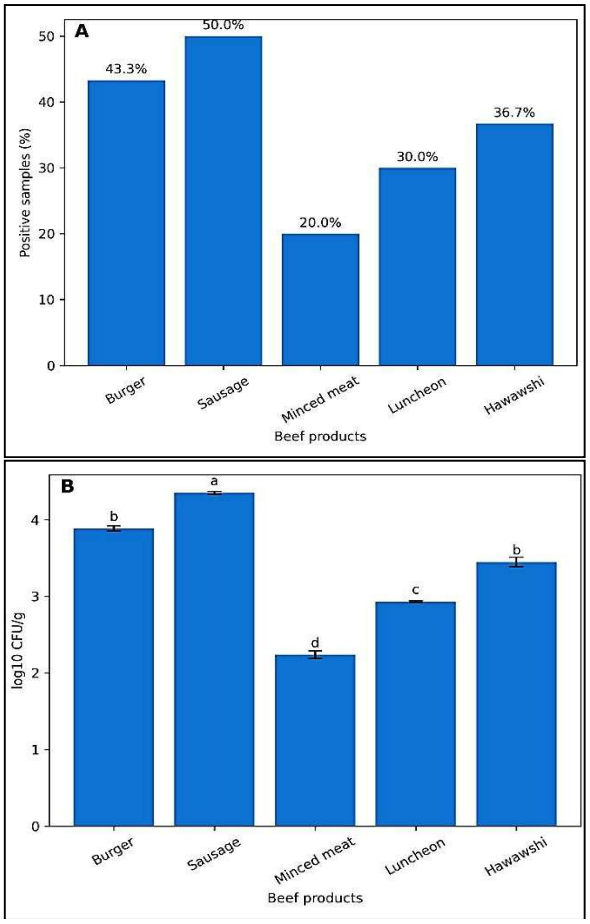

Fig. 1: (A) Percentage of yeast and mold positive samples. (B) Yeast and mold counts expressed as $\log_{10}$ CFU/g (mean±SE). Different letters (a–d) indicate significant differences (P<0.05).

Occurrence of Aflatoxins and Ochratoxin A in Meat Products: Aflatoxins were detected in 46 out of 150 samples (30.7%). The highest occurrence of AFLs was recorded in sausage samples (43.3%; 13/30), followed by burger samples (36.7%; 11/30) and hawawshi (30.0%; 9/30). Lower detection rates were observed in luncheon (26.7%; 8/30) and minced meat (16.7%; 5/30).

Ochratoxin A was detected in 34 out of 150 samples (22.7%). Similar to AFLs, the highest prevalence of OTA was observed in sausage samples (33.3%; 10/30), followed by burger samples (30.0%; 9/30) and hawawshi (23.3%; 7/30). Lower contamination levels were found in luncheon (16.7%; 5/30) and minced meat (10.0%; 3/30).

Overall, sausage and burger samples exhibited the highest contamination rates for both AFLs and OTA, whereas minced meat showed the lowest incidence. "Chi-square analysis revealed no significant differences in the prevalence of AFLs ($\chi^2=5.77$, $p=0.22$) or OTA ($\chi^2=6.24$, $p=0.18$) among the examined beef products (Fig. 2).

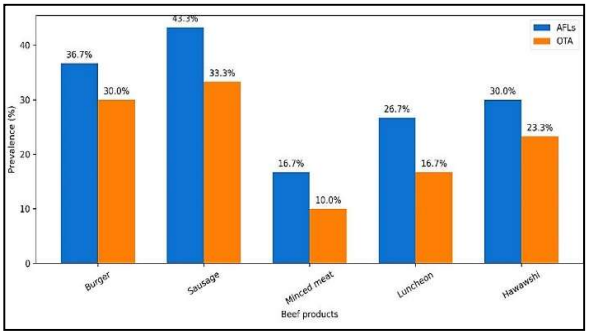

Fig. 2: Prevalence (%) of aflatoxins (AFLs) and ochratoxin A (OTA) in examined beef products. No significant differences were observed among product types (AFLs: χ^2=5.77, p=0.22; OTA: χ^2=6.24, p=0.18).

Total Aflatoxin and Ochratoxin A residues (ppb): Aflatoxin residues in the examined beef products ranged from 2.38 to 25.47ppb (Fig. 3). Sausage samples exhibited the highest mean concentration (16.38 ± 3.65ppb), followed by burger (13.52±3.75), hawawshi (11.54 ±2.65), and luncheon (9.26 ±2.33), while minced meat showed the lowest levels (5.76± .52ppb). Significant differences were observed among product types (P<0.05). Overall, 85.3% of samples were within acceptable limits, whereas 14.7% exceeded the permissible level (20ppb), with the highest non-compliance recorded in sausage samples (23.3%).

Ochratoxin A residues in the examined beef products ranged from 0.54 to 6.33ppb (Fig. 3). Sausage samples showed the highest mean concentration (3.72±0.82ppb), while minced meat exhibited the lowest levels (0.86±0.23ppb). Significant differences were observed among product types (P<0.05). Overall, 95.3% of samples were within acceptable limits, with only 4.7% exceeding recommended levels.

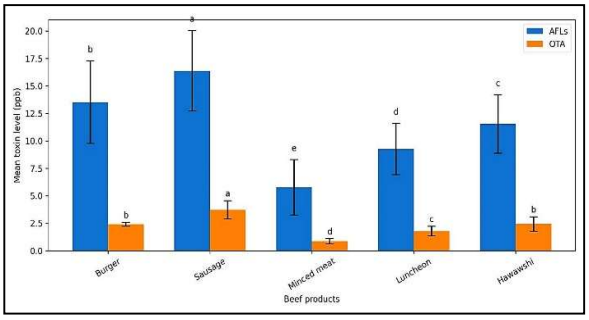

Fig. 3: Comparison of mean aflatoxin (AFLs) and ochratoxin A (OTA) levels (ppb) in examined beef products (mean±SE). Different letters indicate significant differences (p< 0.05) among products within each toxin group.

Correlation between yeast & mold counts and mycotoxin levels in examined beef products: A significant positive correlation was observed between yeast and mold counts and mycotoxin levels in the examined beef products. Yeast and mold counts were moderately correlated with aflatoxin concentrations (r=0.68, p<0.01) and ochratoxin A levels (r=0.61, p<0.05). These results indicate that fungal load and mycotoxin contamination are correlated with the proliferation of fungal toxins in production (Fig. 4).

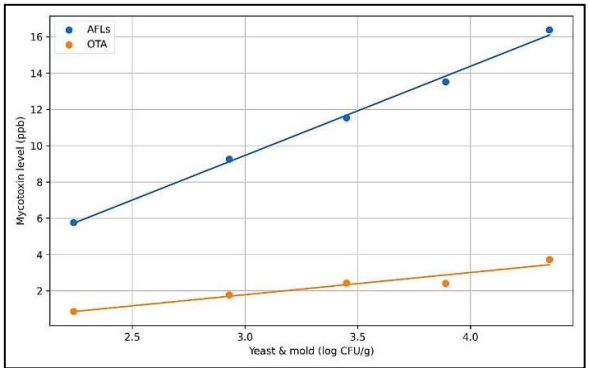

Fig. 4: Correlation of yeast and mold counts (log CFU/g) and of mycotoxin levels (ppb) in analyzed beef products. Both for aflatoxins (AFLs) and ochratoxin A (OTA), a positive connection was found, suggesting that the stronger the fungal infection, the higher the mycotoxin levels.

Effect of laccase concentration on aflatoxin and Ochratoxin A degradation: Untreated control samples showed only a small decrease in aflatoxin levels during storage, from 17.6 ± 0.4 to 15.7 ± 0.5ppb over 15 days. In contrast, the laccase-treated groups showed a clear decrease in AFLs with increasing concentration. The largest degradation was observed in the L4 group (4%), where AFL levels decreased considerably from 17.1±0.2 to 1.7±0.1ppb by the 15th day. Intermediate decreases were observed in the L3, L2, and L1 groups, with a strong association with dosage. Significant differences were observed between all treatments at each storage period (P<0.05), and higher laccase concentrations were always associated with higher detoxification efficiency (Fig. 5).

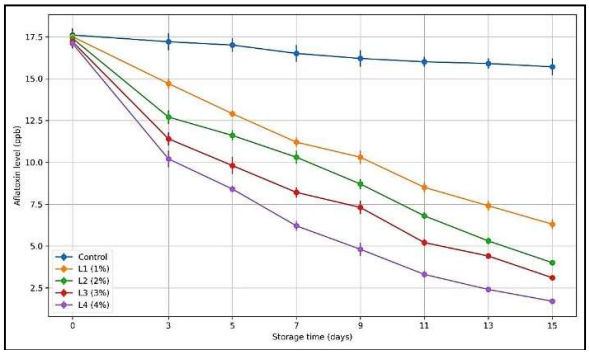

Fig. 5: Influence of laccase content on degradation of aflatoxins (AFLs) in beef samples during refrigerated storage (4°C). Values are reported as mean±SE. The increase of laccase content from 1 to 4% was responsible for a considerable dose-dependent reduction of AFLs over time.

Concerning the amount of Ochratoxin A, control samples exhibited a small decline after storage at refrigeration temperature, from 8.6±0.3 to 7.1±0.2ppb over 15days. Laccase-treated samples, however, displayed a considerable reduction of OTA levels, which was dependent on the concentration. The L4 group showed the largest drop (4%) on day 15, with OTA levels decreasing from 8.1±0.3 to 1.1±0.1ppb. In the L3, L2, and L1 groups, intermediate decreases were observed, demonstrating a strong dosage-response association. Significant changes (p<0.05) were found between all treatments at each storage interval (Fig. 6).

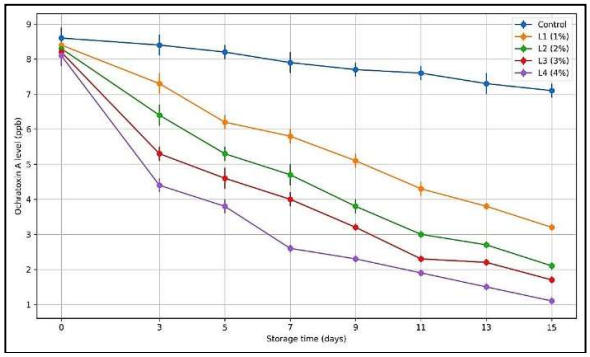

Fig. 6: Influence of laccase concentration on ochratoxin A (OTA) degradation in burger samples experimentally infected and stored at refrigeration temperature (4°C). Values are provided as mean±SD. The laccase concentration was increased (1–4%), which led to a considerable dose-dependent reduction of OTA levels.

The AFLs in laccase-treated samples decreased from 64.0% to 90.1%, with the greatest reduction observed at 4% laccase concentration. OTA also demonstrated reductions from 61.9% to 86.4%. In comparison, minor decreases were seen in control samples (10.8% for AFLs and 17.4% for OTA), confirming that enzymatic treatment was the main driver of mycotoxin degradation (Fig. 7).

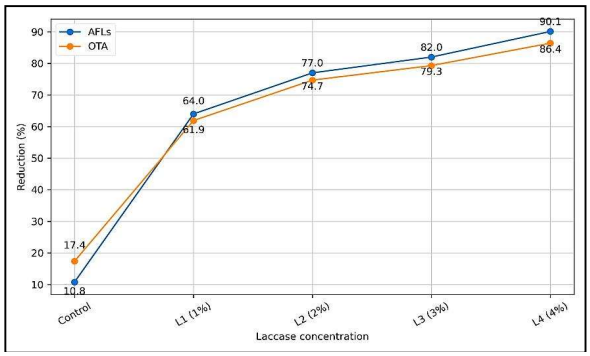

Fig. 7: Percentage reduction of aflatoxins (AFLs) and ochratoxin A (OTA) in experimentally contaminated beef samples treated with escalating amounts of laccase. Both toxins demonstrated a clear dose-dependent decrease, with AFLs showing somewhat better degradation efficiency than OTA.

Degradation Kinetics of Mycotoxins: The breakdown kinetics of aflatoxins (AFLs) and ochratoxin A (OTA) in experimentally contaminated beef burger samples processed with laccase were assessed during refrigerated storage (4°C). The concentrations of the toxins were transformed to ln(Ct/C₀) values to get linear relationships over time for all treatment groups (L1–L4), suggesting that the degradation process followed first-order reaction kinetics (Fig. 8).

The degradation rate was clearly increased in a concentration-dependent manner with increasing amounts of laccase. The rate constant (k) changed from 0.068 day^{-1} (L1) to 0.154 day^{-1} (L4) for AFLs and from 0.064 day^{-1} (L1) to 0.133 day^{-1} (L4) for OTA. This trend confirmed the large increase in detoxifying efficiency with increasing enzyme concentration.

Laccase oxidation was more prone to aflatoxins than OTA, as evidenced by the higher degradation rates of AFLs than OTA in all treatment groups. This discrepancy is likely related to structural differences between the two mycotoxins that affect enzyme-substrate interactions. In contrast, control samples exhibited little drop in toxin levels over time, demonstrating that natural degradation during storage was low and that the observed reductions were primarily due to enzymatic activity.

Overall, the kinetic analysis demonstrates that laccase effectively accelerates mycotoxin degradation in a dose-dependent and time-dependent manner, supporting its potential application as a biotechnological tool for improving the safety of contaminated meat products.

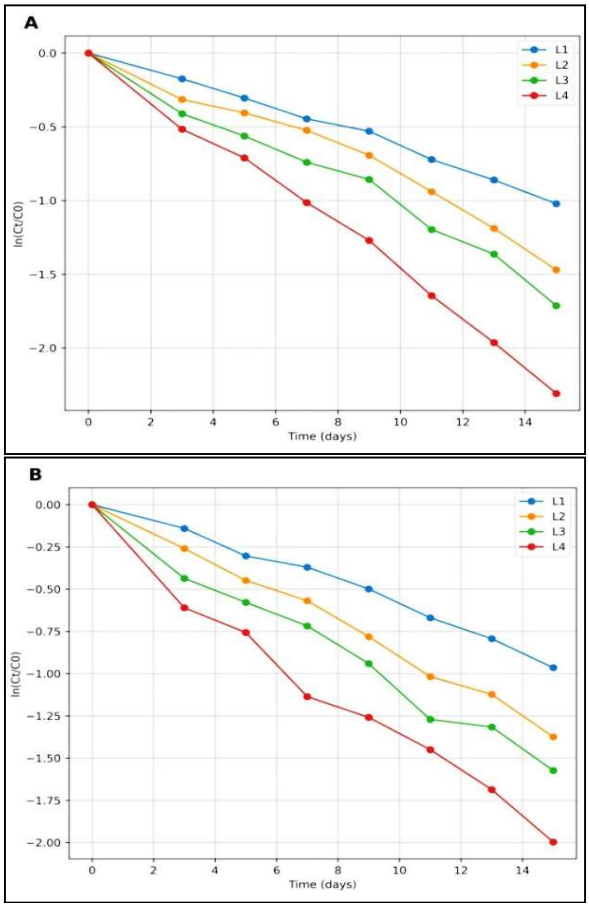

Fig. 8: First-order breakdown kinetics of mycotoxins in experimentally contaminated beef burger sample treated with laccase during refrigerated storage. (A) Aflatoxins (AFLs) and (B) Ochratoxin A (OTA) as ln (Ct/C0) vs time (days). The linear patterns suggest first-order kinetics, and rising laccase concentrations (L1-L4) result in increased degradation rates.

DISCUSSION

The present investigation demonstrated that yeast and mold contamination was detected in 36% of analyzed beef products, with mean counts ranging from 2.24 to 4.35 log10 CFU/g. The highest fungal load was found in sausage samples, followed by burger and hawawshi, while minced meat had the lowest level. These findings offer valuable insights into the microbiological integrity of processed meat products and their potential as vectors for spoilage fungi and mycotoxin-producing species (Gomaa *et al.*, 2024).

The measured contamination levels are comparable to prior data on fungal burdens in meat and meat products.

Khairy *et al*. (2023) showed mold prevalence of 40%, 30%, 30%, and 60% in minced meat, beef burgers, beef lunches, and beef sausages, respectively, with mean mold counts of 2.56±0.02, 2.31±0.86, 2.18±0.87, and 2.50±0.17$\log_{10}$ CFU/g, respectively. The relatively moderate counts recorded in the present work suggest acceptable but not optimal hygienic conditions during processing and retail handling. For instance, yeast and mold counts in beef products were reported to range between 2.9–3.3 $\log_{10}$ CFU/g in retail samples (Yun *et al*., 2025), which is comparable to the levels recorded in minced meat and luncheon in the current study. Similarly, other studies on meat processing environments have shown that fungal counts may reach approximately 4.3–4.5$\log_{10}$ CFU/g during processing stages (Pniewski *et al*., 2026), aligning closely with the elevated counts observed in sausage samples (4.35$\log_{10}$ CFU/g). These similarities indicate that the contamination levels detected in the present study fall within the commonly reported range for meat products under typical retail and processing conditions (Qenawy *et al*., 2024).

The higher fungal counts in sausage and burger samples compared to minced beef could be due to several factors. Sausages and other processed meats can contain a variety of substances (spices, additives, and fillers) that may also serve as sources of further microbial contamination. Furthermore, the procedures involved in handling, grinding, and mixing increase the surface area and exposure to environmental pollutants (Abdelhaseib *et al*., 2025). Recent assessments reinforce this, stating that meat can be contaminated at several stages, including processing, handling, and contact with equipment (Powell *et al*., 2025). In contrast, minced beef revealed lower contamination levels in this investigation, which may be due to shorter processing chains or more recent preparation.

Another key aspect affecting the proliferation of fungi is the inherent nature of animal products. Meat is a nutrient-rich matrix with pH, water activity, and organic substrates that are ideal for the growth of yeasts and molds (Savini *et al*., 2024). However, variation may occur between product types due to differences in formulation, preservation methods, and storage conditions. For instance, heat-treated luncheon meat had lower counts than raw or semi-processed items, demonstrating the efficacy of thermal processing in reducing fungal contamination (Korashi *et al*., 2025).

The presence of yeasts and moulds in meat products, from the point of view of food safety, is of special concern due to their possible role in spoilage and the development of mycotoxins. Fungal contamination can cause sensory deterioration (off-odors, discolouration) and may also contribute to the accumulation of harmful metabolites such as AFLs and OTA (Elbarbary *et al*., 2025). The levels observed in this study emphasize the need to apply rigorous hygienic measures such as good manufacturing practices (GMP), hazard analysis and critical control point (HACCP) systems, and enough refrigeration during storage and distribution (Mohamed *et al*., 2025).

The present study demonstrated that AFLs and OTA were detected in 30.7% and 22.7% of examined beef products, respectively, with higher occurrence in sausage and burger samples compared to minced meat and luncheon. These findings are generally consistent with recent global reports indicating that mycotoxin contamination in meat and meat products is not uncommon. A recent meta-analysis reported an overall OTA prevalence of approximately 39% in meat and meat products, which is higher but comparable to the levels observed in the current study (Sharafi *et al*., 2023). Similarly, aflatoxins have been widely detected in animal-derived products, primarily due to the carryover from contaminated feed or introduction during processing (Syraji *et al*., 2025). Recent surveys have reported the occurrence of AFLs in 60%, 80%, 60%, and 100% of examined minced meat, burgers, luncheon, and sausages, respectively (Elbarbary *et al*., 2023). Similarly, Elbarbary *et al*. (2025) detected AFLs in 86.7%, 60%, 80%, and 70%, while OTA in 83.3%, 56.7%, 80%, and 76.7% of burger, minced beef, luncheon, and sausage samples, respectively. In contrast, Karmi (2019) reported higher contamination rates, with AFLs detected in 96%, 92%, and 76%, and OTA in 96%, 80%, and 72% of burger, luncheon, and minced meat samples, respectively.

The relatively higher occurrence of AFLs and OTA in processed products such as sausage and burger may be attributed to the incorporation of contaminated non-meat ingredients, particularly spices and additives, which are well-known sources of mycotoxins. Additionally, extensive handling and processing steps increase the risk of fungal contamination and subsequent toxin production. Previous studies have confirmed that mycotoxins in meat products can originate either indirectly through contaminated animal feed or directly from fungal growth on product surfaces and added ingredients (Ulusoy *et al*., 2023). This explains the higher contamination rates observed in composite products compared to minced meat, which undergoes fewer processing steps.

The decreased prevalence in minced meat and luncheon may be due to less processing or heat treatment, especially in luncheon meat, which can help prevent fungal growth and toxin production; however, the discovery of mycotoxins in heat-treated products points to their thermal stability and persistence during processing. Aflatoxins and OTA are known to be relatively resistant to standard food processing settings, so they can be found in the final goods even after cooking or preservation (Nazareth *et al*., 2023). This highlights the need to control contamination at early stages of the production chain rather than relying on the processing interventions (Malak *et al*., 2024).

While numerical disparities among beef products were identified, the non-statistically significant differences ($p>0.05$) suggest a broad range of mycotoxin contamination across all beef items tested. This identifies common causes of contamination, such as raw materials, environmental exposure, and storage conditions. Recent studies have highlighted climate factors, insufficient storage, and poor hygiene practices as critical determinants for the ubiquitous presence of mycotoxins, especially in developing nations (Syraji *et al*., 2025).

The elevated levels of AFLs in sausage and burger samples may be attributable to the use of contaminated additives, such as spices, which are known to be important sources of aflatoxins. Moreover, various processing procedures, such as grinding and mixing, increase exposure to fungal contamination (Balan *et al*., 2024). Recent research indicates that aflatoxins are ubiquitous pollutants produced by Aspergillus spp. particularly under favorable

storage circumstances and may be present throughout the food chain (Kępka-Borkowska *et al*., 2025). Furthermore, indirect contamination via animal feed is of key importance, as aflatoxins can be transmitted and accumulate in animal tissues and products (Popescu *et al*., 2022), which explains their presence even in products without apparent fungal growth.

Despite relatively high mean values, the majority of samples (85.3%) were within acceptable limits, while 14.7% exceeded permissible levels, indicating a moderate public health concern. Regulatory limits for aflatoxins vary among international bodies and food categories. While Codex Alimentarius and FAO/WHO standards have set maximum limits for aflatoxins in several food commodities, specific regulatory limits for processed meat products are either restricted or poorly defined in many countries. However, the relatively high AFL levels observed in several sausage and burger samples in the present study may raise food safety concerns, especially regarding cumulative dietary exposure to mycotoxins from different food sources. Similar results were reported in recent research that found that a fraction of beef products may exceed safety limits due to improper storage or contaminated ingredients, particularly in emerging nations with warm climates that favor fungal growth (Aydin, 2020). A high prevalence of AFLs has been reported in certain beef products in previous studies. Elbarbary *et al*. (2023) stated that the mean AFLs residues were 3.9±0.28, 5.4±0.13, 5.14±0.18, and 9.85±0.64ppb in minced meat, beef burger, beef luncheon, and beef sausage, respectively. In the same way, Elbarbary *et al*. (2025) indicated the highest mean levels of AFLs in burgers (13.89±2.62ppb), then sausages (12.67±2.37ppb), luncheon (11.26±2.72ppb), and minced beef (5.47±1.55ppb).

In the case of luncheon, less sophisticated processing and heat treatment may be responsible for lower levels of AFLs in minced beef and luncheon samples. However, the presence of aflatoxins across all product categories demonstrates their strong stability and resilience to traditional processing procedures. Aflatoxins are resistant to common cooking and preservation conditions, which results in persistent health hazards even at low levels of exposure (Nazareth *et al*., 2024).

The present results indicated that OTA levels in the analyzed beef items ranged from 0.54 to 6.33ppb, and the average levels in sausage and burger were higher than those in minced meat and luncheon. The obtained values correspond in general agreement with the previous research revealing OTA values in burgers (2.64±0.14ppb), sausages (2.32±0.57ppb), lunches (2.76±0.43ppb), and minced beef (1.56±0.12ppb) (Elbarbary *et al*., 2025). This agreement indicates that the observed OTA levels are within the common range worldwide, particularly in items with various sources of contamination. Karmi (2019) also found relatively low quantities, with AFLs and OTA levels of 2.7 and 1.04ppb in burgers, 2.3 and 1.4ppb in lunches, and 2.5 and 1.03ppb in minced beef, respectively.

The elevated OTA levels in processed products such as sausage and burgers can be attributed to both carryover from contaminated animal feed and contamination after processing from components and surroundings. OTA is mostly consumed through contaminated feed and may bioaccumulate in animal tissues due to its high bioavailability and delayed clearance rate (Ganesan *et al*., 2021). Processed meat products also typically contain spices and chemicals, which are known sources of OTA contamination. Recent studies indicate that OTA is widely detected in various food commodities, such as processed meats, due to fungal growth under favorable storage conditions (Ben Miri *et al*., 2024). These factors are probably responsible for the substantially greater amounts observed in composite meat products compared with simpler matrices such as minced meat (Khairy *et al*., 2024).

The majority of samples (95.3%) were within acceptable limits, even in the presence of OTA; only 4.7% exceeded the allowable levels, indicating a rather excellent overall safety. However, several of the discovered values, particularly in sausage, were close to the recommended limits (5ppb) set by the FAO/WHO (2001) and Codex Alimentarius (2019), which is worth noting and raises health concerns. The presence of OTA in heat-processed items, such as luncheon, further shows its excellent thermal stability as it is resistant to typical food processing and preservation procedures .

The results of the present investigation showed a favorable association between yeast and mold counts and mycotoxin levels, and fungal load was moderately correlated with aflatoxin levels (r=0.68) and ochratoxin A levels (r=0.61). These results are in good agreement with recent literature indicating that mycotoxins are secondary metabolites produced directly by toxigenic fungi, especially Aspergillus, Penicillium, and Fusarium species, and that their levels tend to increase with fungal growth (Nazareth *et al*., 2024). Similarly, recent research has shown that aflatoxin incidence is strongly associated with the presence and activity of aflatoxigenic fungi, with a high proportion of isolates capable of producing toxin (Gherbawy *et al*., 2025).

Recent reviews highlight that the mere presence of fungi is not necessarily a direct predictor of toxin levels, as toxin production is dependent on specific growth conditions and gene expression of toxigenic strains (Syraji *et al*., 2025). Studies also reveal common co-contamination and interactions among different fungi and toxins that may contribute to the unpredictability of correlation intensity (Pokoo-Aikins *et al*., 2024). The observed results are in line with the idea that fungal contamination is a major factor in mycotoxin occurrence. Control of yeast and mold development is an important technique to reduce mycotoxin hazards in meat products (Khairy *et al*., 2024).

Laccase, a multicopper oxidase enzyme, is broadly generated by fungi and able to oxidize a wide spectrum of phenolic and non-phenolic substances. Due to its significant oxidative potential (Zhao *et al*., 2025), laccase has attracted substantial attention as a green and effective biocatalyst for the detoxification of mycotoxins, particularly aflatoxins, in food systems.

The present investigation indicated that recombinant laccase effectively lowered the levels of both AFLs and OTA in experimentally contaminated burger samples, in a dose- and time-dependent manner during refrigerated storage. The control samples showed only minor natural reductions, whereas all treatment groups had considerable reductions in toxin levels. The maximum detoxification was reported at 4% laccase (L4) by day 15. Importantly, the

degradation of aflatoxins was more evident than that of OTA, in agreement with differences in their chemical structures and sensitivity to enzymatic oxidation. This is in accordance with recent research demonstrating the efficiency of laccase enzymes as biocatalysts for the breakdown of aflatoxin under mild conditions. For instance, laccase was found to degrade aflatoxins and transform them into less hazardous metabolites, with degradation efficiency increasing over time and with increasing enzyme activity (Hao *et al*., 2025). Another study also showed partial to complete destruction of aflatoxins depending on enzyme concentration and incubation conditions, corroborating the dose-dependent impact seen in the current work (Loi *et al*., 2023).

The observed detoxifying effect is ascribed to the oxidative catalytic activity of laccase, which acts on the chemical structure of mycotoxins, breaking them down into less toxic molecules. The greater reduction of AFLs than of OTA is consistent with earlier observations that OTA is considerably more resistant to enzymatic degradation due to its more stable molecular structure. Recent studies have demonstrated that laccase-based systems can achieve high degradation rates of aflatoxins, whereas OTA degradation is often partial and depends on the enzyme and reaction conditions (Jia *et al*., 2024). However, some investigations also suggest that the substrate structure and enzyme affinity may impede complete breakdown, which may explain the detection of residual toxin even at the maximum treatment level (Zaccaria *et al*., 2023). Moreover, the continuous decrease in both toxins during storage demonstrates that laccase remains active under chilled conditions, a very important feature for its application in real food matrices.

The present investigation of degradation kinetics showed that the detoxification of both AFLs and OTA mediated by laccase is time- and concentration-dependent, with a rapid initial decrease followed by a gradual decrease with prolonged storage. This trend is consistent with previously described models of enzymatic degradation for oxidative enzymes, in which detoxification often follows pseudo-first-order kinetics under substrate-limited conditions (Zhao *et al*. 2025).

The detoxifying mechanism of laccase is mainly due to the one-electron oxidation reaction catalyzed by the multicopper oxidase system, which produces reactive radical intermediates and reduces molecular oxygen to water. Laccase is thought to attack the highly reactive double bond in the terminal furan ring of aflatoxins, resulting in oxidative cleavage and structural instability of the toxin molecule. This alteration decreases the electrophilic activity, which causes the toxicity and mutagenicity of aflatoxins. OTA degradation may include oxidation of the phenolic moiety and cleavage of the isocoumarin structure, leading to decreased toxin stability and biological action (Hao *et al*., 2023).

The more rapid breakdown of AFLs compared to OTA may be associated with structural variations between the two toxins and their respective vulnerability to oxidative assault by laccase-generated radicals. Also, the decline in degradation rate over time may be related to a loss of substrate supply, partial enzyme inactivation during storage, or accumulation of intermediate oxidation products that may interfere with enzyme action. Recent investigations have demonstrated similar oxidative degradation pathways for laccase-mediated mycotoxin detoxification (Loi *et al*., 2023).

In general, the current results are consistent with the recent literature, which emphasizes enzymatic detoxification as an environmentally friendly and promising approach to managing mycotoxins in food systems. For example, evaluations published highlight the many benefits of laccase, such as its specificity, minimal impact on food quality, and ability to operate under moderate conditions (Liu *et al*., 2024). Nevertheless, the residual levels of toxin persistence, especially for OTA, indicate that full detoxification may be achieved by optimizing the enzyme concentration, using mediators, or combining with other detoxification techniques. However, the dose-dependent response and sustained activity seen in this investigation confirm the high potential of recombinant laccase as a useful tool for lowering mycotoxin contamination in meat products (Aasa *et al*., 2026).

Study Limitations: Despite the promising findings, several limitations should be acknowledged. First, the study was conducted under controlled conditions using artificially contaminated burger samples, which may not fully represent the complexity of naturally contaminated meat products. Second, although laccase efficiently degraded AFLs and OTA, the degradation products were not identified, and the safety of the resulting metabolites was not evaluated. Therefore, future studies should employ advanced analytical techniques, such as LC–MS/MS or HRMS, to characterize degradation intermediates and confirm the safety of detoxification.

In addition, the study was limited to refrigerated storage conditions. It did not assess the effects of other factors, such as pH, temperature fluctuations, oxygen availability, or packaging systems, on enzyme activity and degradation efficiency. The tested laccase concentrations were also restricted to 1–4%, without broader optimization of enzyme dose or contact time.

Furthermore, the potential effects of laccase treatment on the sensory, physicochemical, and nutritional quality of meat products were not investigated. Future studies should therefore include sensory evaluation, texture and color analysis, lipid oxidation assessment, and consumer acceptability testing, in addition to validation under industrial processing and storage conditions.

Conclusions: The present investigation showed the presence of fungal contamination and associated mycotoxins, such as AFLs and OTA, in commonly consumed beef products, with higher levels in processed goods like sausages and burgers. The strong association between yeast and mold counts and mycotoxin levels indicates the crucial role of fungal contamination in toxin formation. It underscores the importance of improved sanitary procedures during processing and storage. In addition, recombinant laccase treatment resulted in a significant dose- and time-dependent decrease in both AFLs and OTA in experimentally contaminated burger samples under refrigerated storage conditions. AFLs were more susceptible to enzymatic degradation than OTA. These results imply that recombinant laccase can be an environmentally benign strategy for mycotoxin reduction in meat systems. Nevertheless, to apply this approach in

practice, further studies are necessary to determine the degradation products, optimize treatment conditions, evaluate sensory and quality attributes, and validate its effectiveness and feasibility under industrial processing conditions.

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