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Unmasking Mycotoxins: Effects of Sodium Hypochlorite Sterilisation and Autoclaving on Zearalenone Contamination in Grain Corn

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ABSTRACT

This study investigates the effects of sodium hypochlorite (NaOCl) and autoclaving on zearalenone (ZEA), a *Fusarium*-derived mycotoxin in grain corn, to optimize sample preparation for biosensor analysis. ZEA often exists in bound forms, complicating detection. Three grain corn samples—freshly harvested (IC3924), stored for one month (C1M), and two months (C2M) were treated with 30% NaOCl alone or followed by autoclaving. A phosphate-buffered saline (0.01 M) served as a control, resulting in 10 samples. ZEA levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Untreated C1M and IC3924 showed no detectable ZEA, while untreated C2M contained 4.89 ppb. Sodium hypochlorite (NaOCl) alone eliminated detectable ZEA, but autoclaving increased its levels, likely due to bound toxin release under heat and pressure. This suggests autoclaving may artificially elevate ZEA detection, potentially affecting food safety assessments. In contrast, NaOCl treatment alone effectively removes detectable ZEA. These findings underscore the importance of optimizing sample preparation for accurate mycotoxin detection in grain corn, particularly for biosensor applications.

Keywords: Autoclaving, grain corn, masked mycotoxins, sterilisation, zearalenone

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INTRODUCTION

Mycotoxin contamination in agricultural commodities poses significant challenges to food safety, public health, and economic stability. Zearalenone (ZEA), a *Fusarium*-derived mycotoxin, is particularly

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concerning due to its estrogenic properties and associated health risks in humans and livestock. Grain corn, a staple in food and feed, is highly vulnerable to *Fusarium* contamination under warm, humid conditions (Qu et al., 2024). ZEA mimics estrogen, causing reproductive disorders in livestock and raising concerns about endocrine disruption in humans, necessitating strict regulatory limits (Berthiller et al., 2009). Additionally, ZEA can exist in free or bound (masked) forms, complicating detection and potentially underestimating contamination levels.

Sterilisation methods like autoclaving and chemical treatments may alter ZEA's structure, degrade it, or release bound forms, affecting quantification (Ibáñez-Vea et al., 2012). However, systematic studies on their impact on ZEA derivatives are limited. This study evaluates the effects of autoclaving and sodium hypochlorite (NaOCl) on ZEA in grain corn, aims to establish an optimized sample preparation method for electrochemical biosensor-based detection. By analyzing ZEA levels in freshly harvested and stored corn under different treatments, this research provides insights into pre-analytical processing for mycotoxin detection. The findings will advance biosensing platforms, improving food safety monitoring and regulatory compliance in agriculture.

MATERIALS AND METHODS

Harvested grain corn samples were obtained from the Industrial Crop Research Centre, MARDI, Malaysia. The samples used in this study were of the P4546 variety from DuPont, Thailand, cultivated at MARDI Seberang Perai, Penang, Malaysia. Three sample batches were tested: freshly harvested grain corn (IC3924), grain corn stored for one month (C1M), and grain corn stored for two months (C2M). Each batch was subjected to either 30% sodium hypochlorite (NaOCl) treatment alone or NaOCl treatment followed by autoclaving at 121°C for 15 min. In total, 10 samples were analyzed, including a phosphate-buffered saline (0.01 M) solution as a negative control. A commercial RIDASCREEN® FAST Zearalenone kit (R5502) was utilized in this study to quantify the amount of zearalenone mycotoxin in the samples.

RESULTS AND DISCUSSION

As presented in Table 1, results indicate that untreated C1M and IC3924 samples had no detectable zearalenone, while untreated C2M contained 4.89 ppb, suggesting prolonged storage may increase contamination risk. Autoclaved samples exhibited higher zearalenone levels, with IC3924-CA (1.29 ppb), C1M-CA (2.86 ppb), and C2M-CA (6.42 ppb), This rise is due to the release of bound or masked zearalenone during autoclaving, a phenomenon documented in mycotoxin research (Gratz, 2017). Under condition of high heat and pressure, bound mycotoxinsconjugated with grain cellular components may be liberated resulting in increasing measured concentrations. Autoclaving may also enhance extraction efficiency, potentially resulting in artificially elevated detection levels.

Table 1
Zearalenone concentration (ppb) in grain corn samples under different sterilisation treatments

Samples	Treatment	Zearalenone (ppb)
0.01 M PBS	(negative control)	Not detected
IC3924	Untreated	Not detected
C1M	Untreated	Not detected
C2M	Untreated	4.89
IC3924-C	Sterile with 30% NaOCl	Not detected
C1M-C	Sterile with 30% NaOCl	Not detected
C2M-C	Sterile with 30% NaOCl	Not detected
IC3924-CA	30% NaOCl and autoclaved	1.29
C1M-CA	30% NaOCl and autoclaved	2.86
C2M-CA	30% NaOCl and autoclaved	6.42

While heat treatment can degrade certain mycotoxins, *Fusarium*-derived toxins like zearalenone and deoxynivalenol (DON) may form new derivatives during thermal processing, potentially altering toxicity or enhancing detectability (Karlovsky et al., 2016). This underscores the complex effect of autoclaving on mycotoxin quantification and food safety assessments. In contrast, samples treated with NaOCl alone showed no detectable zearalenone, indicating that sodium hypochlorite effectively neutralizes or removes the mycotoxin before further processing. This finding aligns with previous studies on the efficacy of chlorine-based disinfectants in reducing mycotoxin contamination in food matrices (Nešić et al., 2023).

These findings highlight the importance of selecting appropriate matrix treatment methods for preparing sterile materials in zearalenone standard curve development, ensuring accuracy in biosensor applications.

CONCLUSION

The study emphasizes the need to consider the effects of storage, chemical treatment, and autoclaving on mycotoxin detection to avoid misinterpretation of analytical results. These findings indicate that autoclaving releases bound zearalenone, increasing detection levels, while sodium hypochlorite (NaOCl) treatment effectively eliminates detectable contamination. For future zearalenone biosensor development, NaOCl treatment will be employed, with C1M selected as the primary sample type to ensure controlled and accurate mycotoxin assessment.

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REFERENCES

Berthiller, F., Schuhmacher, R., Adam, G., & Krska, R. (2009). Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry, 395*(5), 1243-1252. https://doi.org/10.1007/s00216-009-2874-x

Karlovsky, P., Suman, M., Berthiller, F., De Meester, J., Eisenbrand, G., Perrin, I., Oswald, I.P., Speijers, G., Chiodini, A., Recker, T., & Dussort, P. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research, 32*(4), 179-205. https://doi.org/10.1007/s12550-016-0257-7

Ibáñez-Vea, M., González-Peñas, E., Lizarraga, E., & De Cerain, A. L. (2012). Co-occurrence of aflatoxins, ochratoxin A and zearalenone in barley from a northern region of Spain. *Food Chemistry, 132*(1), 35-42. https://doi.org/10.1016/j.foodchem.2011.10.023

Gratz, S. W. (2017). Do plant-bound masked mycotoxins contribute to toxicity? *Toxins, 9*(3), Article 85. https://doi.org/10.3390/toxins9030085

Nešić, K., Habschied, K., & Mastanjević, K. (2023). Modified mycotoxins and multitoxin contamination of food and feed as major analytical challenges. *Toxins, 15*(8), Article 511. https://doi.org/10.3390/toxins15080511

Qu, Z., Ren, X., Du, Z., Hou, J., Li, Y., Yao, Y., & An, Y. (2024). *Fusarium* mycotoxins: The major food contaminants. *Mlife, 3*(2), 176-206. https://doi.org/10.1002/mlf2.12112