Volume (6) Issue (3)

25 Aug 2026

The International Journal of Scientific Publishing

المجلة الدولية للنشر العلمي

Mycological Quality of Marketed Cheese and Screening for Aflatoxin M1 and Ochratoxin A in El-Bayda City, Libya

Marwa R. Ahmaida – Shadia Awad

(Department of Food Health Control, Faculty of Veterinary Medicine, Omar Al-Mukhtar University, Al-Beyda, Libya)

https://doi.org/10.65723/RMSP3069

Abstract:

Cheese is susceptible to fungal spoilage and mycotoxin contamination, which may originate from contaminated feed, the production environment, or storage. This cross-sectional market survey evaluated the mycological quality of 30 cheese samples (15 soft and 15 hard) collected in El-Bayda City, Libya, and screened samples for aflatoxin M1 (AFM1) and ochratoxin A (OTA). The reported mean mould and yeast count was higher in soft cheese ($4.2 \times 10^4 \pm 0.20 \times 10^4$ CFU/g) than in hard cheese ($2.8 \times 10^4 \pm 0.10 \times 10^4$ CFU/g). Among 30 recorded primary fungal isolates, Penicillium spp. accounted for 46.7% (14/30), Aspergillus spp. for 33.3% (10/30), and Mucor spp. for 20.0% (6/30). AFM1 and OTA were reported in 40.0% (12/30) and 26.7% (8/30) of samples, respectively. These findings indicate that fungal contamination and mycotoxin detection merit strengthened surveillance. However, toxin concentrations, detection limits, recovery, individual sample results, and raw colony counts were not available; therefore, this study reports screening frequency and does not assess regulatory compliance, dietary exposure, or causal relationships between fungal genera and toxins. The manuscript recommends validated quantitative mycotoxin methods, source-water/feed and storage control, and a risk-based hygiene programme for dairy products marketed in El-Bayda.

Keywords: cheese; mycological quality; moulds; yeasts; aflatoxin M1; ochratoxin A; food safety; El-Bayda; Libya

1. Introduction

Cheese is a nutrient-dense dairy product but may provide a suitable niche for fungal growth during manufacture, ripening, cutting, packaging, storage, and retail distribution. Fungal contamination can cause visible spoilage, off-flavours, economic loss, and, depending on the fungal species and food matrix, mycotoxin hazards. Important fungi associated with cheese include Penicillium, Aspergillus, Mucor, Cladosporium, and Geotrichum; some are deliberately used in particular cheese styles, whereas uncontrolled contaminants may be undesirable (Hymery et al., 2014; Garnier et al., 2017).

Aflatoxin M1 (AFM1) and ochratoxin A (OTA) have distinct pathways that should not be conflated. AFM1 is a hydroxylated metabolite of aflatoxin B1 that can be excreted in milk after lactating animals consume contaminated feed; it may partition into the curd during cheese making because of its affinity for casein (Iqbal et al., 2015; Aranda et al., 2025). In contrast, OTA in cheese is often considered in relation to environmental fungal growth, especially on rinds and in products exposed to unsuitable storage conditions, although its origin should be demonstrated rather than assumed (Pattono et al., 2013; Pavicich et al., 2024).

Evidence from cheese surveys demonstrates that detection frequencies vary widely across product types, geography, analytical method, and sampling frame. A global meta-analysis estimated pooled prevalence of 49.85% for AFM1 and 35.64% for OTA, but the wide between-study variability means that local estimates need transparent methods and quantitative results (Mahmudiono et al., 2024). In Libya, locally generated data can help identify priority control points, but only when sample origin, analytical performance, and denominator definitions are clearly reported.

The present study aimed to describe the reported mould-and-yeast burden, fungal isolate distribution, and screening positivity for AFM1 and OTA among cheese samples marketed in El-Bayda City. A second aim of this revision was to distinguish the evidence supported by the supplied dataset from conclusions that require additional laboratory and statistical documentation.

2. Materials and Methods

2.1 Study design and sampling:

A cross-sectional market survey was conducted in El-Bayda City, eastern Libya. Thirty cheese samples were collected: 15 soft and 15 hard cheese. Samples were reportedly purchased from supermarkets, local markets, and street vendors, transported aseptically in ice boxes, and analysed within 24 h. Before submission, the authors should add collection dates, product brand/origin, milk type, manufacture/expiry date, packaging status, storage temperature, vendor category, and a sample-identification code.

2.2 Mycological examination:

The supplied protocol states that total mould and yeast counts were determined on Sabouraud dextrose agar at 25 °C for 5–7 days and isolates were identified from macroscopic and microscopic characteristics. To make the method reproducible, the final protocol must specify sample mass, diluent, serial-dilution scheme, plated volume, number of plates, medium formulation/antibiotic use, colony-count selection range, and quality-control strains. Morphology-based identification is reported at genus level only; species-level or molecular identification is necessary to infer toxigenic potential for an individual isolate (Pitt & Hocking, 2009).

2.3 Mycotoxin analysis:

AFM1 was reportedly screened by ELISA and OTA by HPLC. The revised manuscript treats these as screening outcomes because no concentration values or analytical-validation information was supplied. A final laboratory report should state the kit manufacturer and lot, standards, calibration curve, extraction and clean-up, chromatographic column/detector/mobile phase, LOD, LOQ, recovery, precision, matrix effects, and confirmation criteria. Contemporary cheese methods commonly use validated LC-MS/MS or appropriately validated immunoassay/HPLC workflows (Rodríguez-Cañás et al., 2023; Pavicich et al., 2024).

2.4 Statistical analysis:

Descriptive statistics are reported as supplied. Wilson 95% confidence intervals were calculated for the fungal-isolate and toxin-screening proportions (n = 30). The reported independent-samples t statistic and χ^2 values were not interpreted because raw colony counts and relevant contingency tables were unavailable. The supplied mean ± values and t = 5.44 cannot be verified from summary data alone. Future analysis should log-transform CFU/g where appropriate, examine model assumptions, report exact p values and effect sizes, and pre-specify how multiple isolates or toxin co-occurrence are handled.

2.5 Interpretation boundaries:

No AFM1 or OTA concentration, co-occurrence status, or regulatory benchmark comparison was supplied. Thus, the study cannot determine the proportion of samples exceeding any maximum level, calculate exposure, or establish that recovered genera produced the detected toxins. The results are interpreted as evidence that further quantitative surveillance and control are warranted, not as proof of consumer health risk in a particular sample.

3. Results

3.1 Mould and yeast counts: The reported mean mould and yeast count was $4.2 \times 10^4 \pm 0.20 \times 10^4$ CFU/g for soft cheese and $2.8 \times 10^4 \pm 0.10 \times 10^4$ CFU/g for hard cheese (Table 1; Figure 1). Soft cheese therefore showed a higher reported mean count. Because the raw counts were not available and the supplied dispersion term was not defined, this revision does not present an inferential p value. The result should be regarded as a descriptive comparison pending verification from the original laboratory worksheet.

Sample type	n	Reported mean mould/yeast count (CFU/g)	Interpretation
Soft cheese	15	$4.2 \times 10^4 \pm 0.20 \times 10^4$	Higher reported mean; significance not re-tested without raw data.
Hard cheese	15	$2.8 \times 10^4 \pm 0.10 \times 10^4$	Lower reported mean; significance not re-tested without raw data.

Table 1. Reported total mould and yeast counts. The reported t statistic was not retained because raw observations were unavailable.

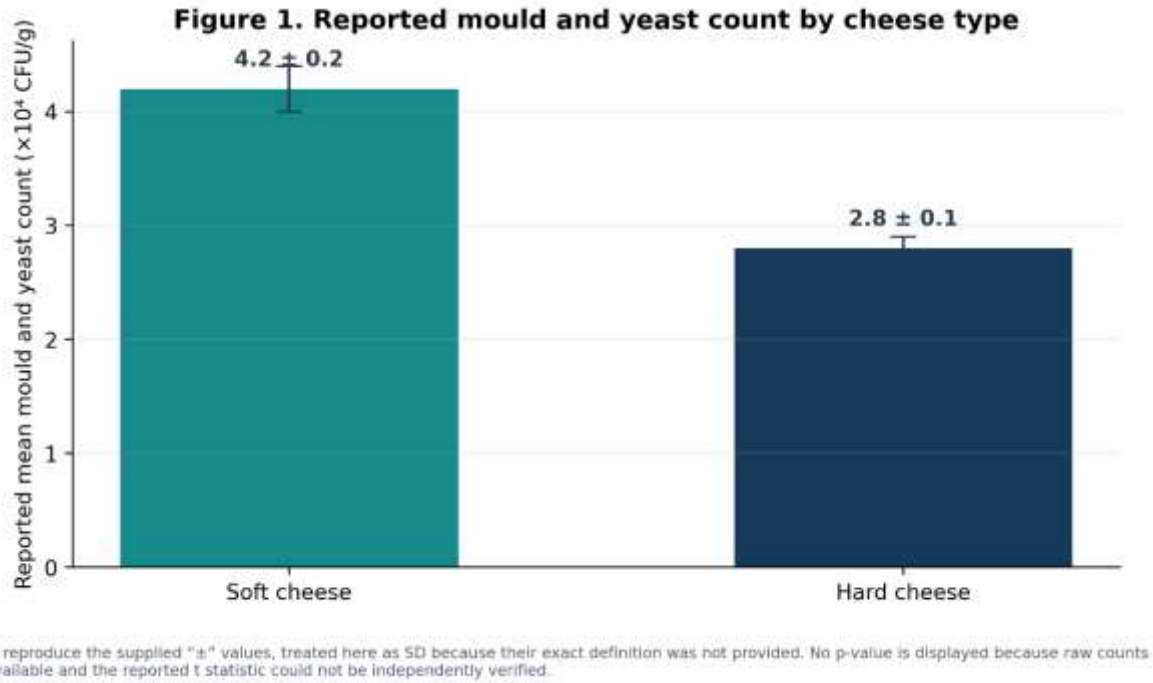

Figure 1. Reported mould and yeast count by cheese type.

3.2 Fungal isolate distribution:

The submitted genus frequencies sum to 30. Under the working assumption that one primary isolate record was retained per sample, Penicillium spp. were the most frequent (14/30; 46.7%; 95% CI 30.2–63.9%), followed by Aspergillus spp. (10/30; 33.3%; 95% CI 19.2–51.2%) and Mucor spp. (6/30; 20.0%; 95% CI 9.5–37.3%) (Table 2; Figure 2). These observations support a need for species-level identification and source tracing but do not establish toxin production by any isolate.

Genus	Frequency	Percentage	95% Wilson CI
Penicillium spp.	14/30	46.7%	30.2–63.9%
Aspergillus spp.	10/30	33.3%	19.2–51.2%
Mucor spp.	6/30	20.0%	9.5–37.3%

Table 2. Frequency of reported fungal genera. Wilson confidence intervals are calculated from n = 30.

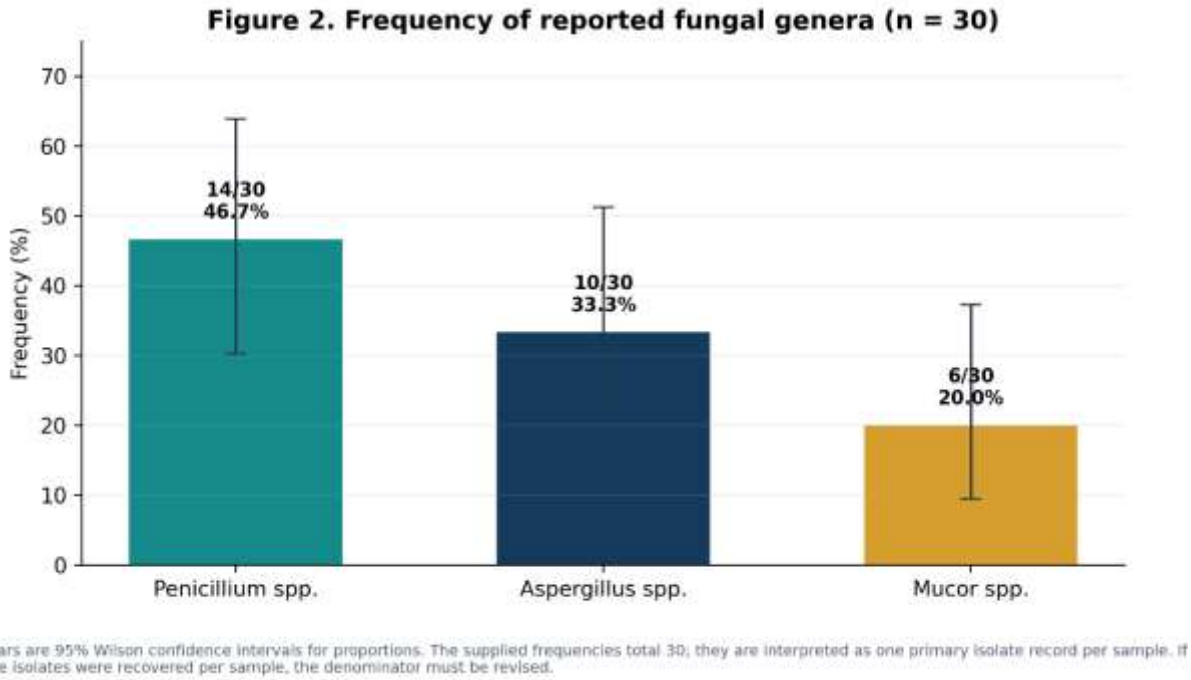

Figure 2. Frequency of reported fungal genera.

3.3 Mycotoxin screening:

AFM1 was reported in 12/30 samples (40.0%; 95% CI 23.2–59.2%) and OTA in 8/30 samples (26.7%; 95% CI 14.2–44.4%) (Table 3; Figure 3). The outcomes are detection frequencies only. Without concentrations, LOD/LOQ, recovery, and information on whether the same samples contained both toxins, no conclusion can be drawn about compliance, exposure, co-occurrence, or comparative prevalence. The supplied $\chi^2 = 7.28$ is not reported because no valid comparison table or null hypothesis was specified.

Mycotoxin	Screen-positive samples	Percentage	95% Wilson CI	What cannot be assessed
AFM1	12/30	40.0%	23.2–59.2%	Concentration, compliance, exposure, source.
OTA	8/30	26.7%	14.2–44.4%	Concentration, compliance, co-occurrence, source.

Table 3. Mycotoxin screening frequency. No concentration or regulatory-compliance conclusion is possible from these data.

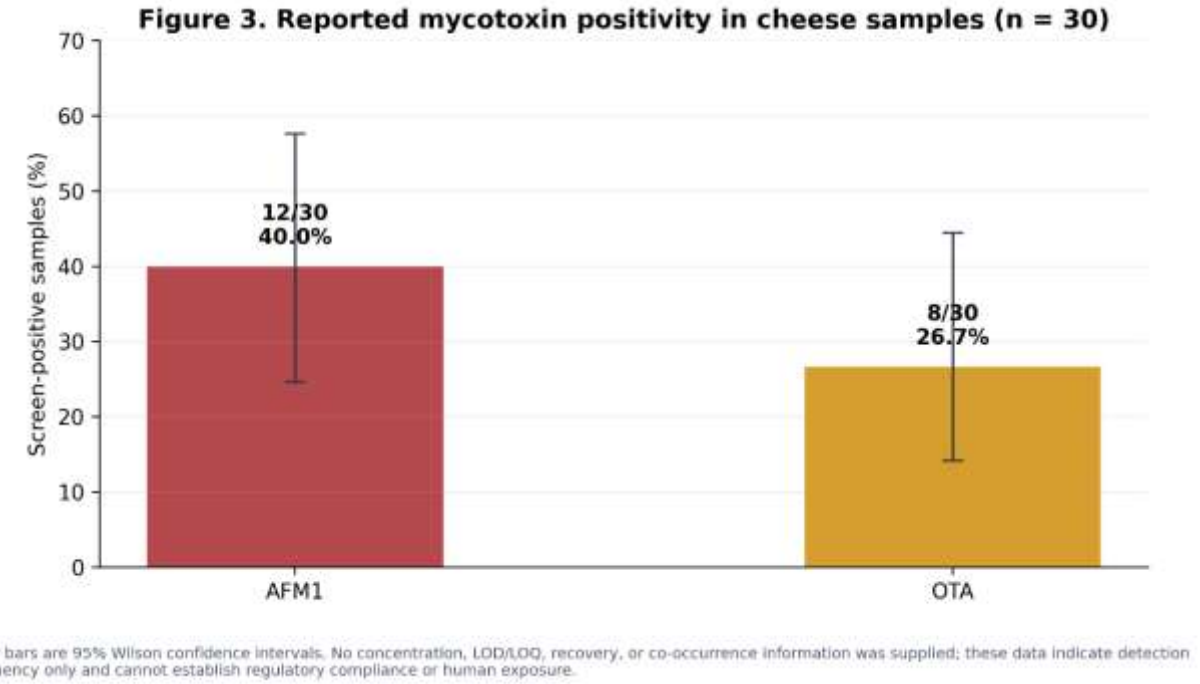

Figure 3. Reported mycotoxin positivity with 95% Wilson confidence intervals.

3.4 Control implications:

The pattern of reported fungal contamination and toxin screening warrants a preventive approach covering dairy-feed control, hygienic manufacture, cold-chain/storage control, and validated laboratory verification (Figure 4). The data do not identify the stage of contamination. AFM1 detection should prompt investigation of milk/feed supply, while any OTA investigation should include storage conditions, cheese rind versus core, fungal species confirmation, and packaging history.

Figure 4. Preventive control and verification pathway for cheese safety.

4. Discussion

The reported higher fungal count in soft cheese is biologically plausible because moisture, water activity, surface handling, and shorter preservation barriers can favour fungal development. Nevertheless, plausibility is not a substitute for a complete product characterization. Moisture, pH, water activity, salt, storage temperature, package integrity, and shelf age were not supplied and should be incorporated in future work to identify why samples differ (Hymery et al., 2014; Garnier et al., 2017).

Penicillium, Aspergillus, and Mucor are all known from dairy environments, but genus-level morphology is insufficient to classify an isolate as toxigenic. Some Penicillium species are legitimate ripening organisms; others are spoilage organisms, and the ability to synthesize a particular metabolite varies by species and strain. The inference that Aspergillus or Penicillium isolated from a sample caused the measured toxin should therefore be avoided unless the isolate is identified and its toxigenic capacity, toxin profile, and spatial relation to the contaminated cheese are demonstrated.

The AFM1 result requires particular care. AFM1 is generally linked to the feed–animal–milk pathway and can be enriched in curd, so its occurrence is not evidence that cheese-surface fungi created AFM1. Conversely, OTA may be associated with fungal contamination of the rind or storage environment, but this source attribution still requires sample-level evidence. Quantitative data are essential: a positive/negative percentage alone cannot establish whether samples exceed a regulatory threshold or pose a defined public-health risk (Iqbal et al., 2015; Mahmudiono et al., 2024).

The investigation is valuable as a local screening study but has limitations: modest sample size, no documented sampling frame or dates, unreported laboratory validation parameters, no concentration data, unverified hypothesis-test values, and no species-level confirmation. These limitations are explicitly retained to protect the credibility of the work. They should be addressed through a larger seasonal survey that samples rind and core separately, uses pre-defined sample codes, records environmental/retail variables, and confirms positive toxin screens using a validated quantitative method.

Practical controls should include feed surveillance for aflatoxin B1, supplier approval, sanitation and zoning in production areas, management of humidity and condensation, refrigeration and shelf-life control, package-integrity checks, and routine environmental mould monitoring. A risk-based programme should distinguish cheeses where fungal growth is intentional from products where it represents spoilage, while preserving traceability from farm to retail.

5. Conclusions

This survey reports higher mean mould and yeast counts in soft than hard cheese and identifies Penicillium spp., Aspergillus spp., and Mucor spp. among the recorded primary isolates. AFM1 and OTA were reported in 40.0% and 26.7% of the 30 cheese samples, respectively. These findings justify stronger food-safety surveillance in El-Bayda.

However, the current dataset supports screening-frequency statements only. It does not support concentration-based regulatory assessment, exposure assessment, source attribution, or the originally supplied t and χ^2 significance claims. A strengthened next study should provide raw CFU/g observations, validated analytical concentration data, species-level fungal identification, rind/core sampling, and an auditable statistical plan. This approach will make future evidence suitable for a peer-reviewed food-safety journal.

Declarations

Ethics approval: Not stated in the source material. Insert institutional approval or exemption details before submission.

Data availability: The supplied summary data, derived confidence intervals, figure values, and reference links are available in Cheese_data_figures_and_reference_log.xlsx. Individual laboratory data were not provided.

Conflicts of interest: The authors declare no conflicts of interest. [Revise if applicable.]

Funding: No funding statement was supplied. [Insert funding information or state "No external funding."]

Author contributions: [Insert actual author contributions using CRediT roles.]

References:

Aranda, C., Rodriguez, R., Fernández-Baldo, M. A., & Durán, P. (2025). Mycotoxins in cheese: Assessing risks, fungal contaminants, and control strategies for food safety. Foods, 14(3), 351. https://doi.org/10.3390/foods14030351

Elramly, M. H., El-Leboudy, A. A., & Al-Ansary, M. A. (2019). Mycological evaluation of Egyptian Ras cheese with special reference to mycotoxins. Alexandria Journal of Veterinary Sciences, 63(2), 33–38. https://doi.org/10.5455/ajvs.58688

Elzupir, A. O., & Elhussein, A. M. (2010). Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan. Food Control, 21(7), 945–946. https://doi.org/10.1016/j.foodcont.2009.11.002

European Commission. (2023). Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food. https://eur-lex.europa.eu/eli/reg/2023/915/oj

Garnier, L., Valence, F., & Mounier, J. (2017). Diversity and control of spoilage fungi in dairy products: An update. Microorganisms, 5(3), 42. https://doi.org/10.3390/microorganisms5030042

Hymery, N., Vasseur, V., Coton, M., Mounier, J., Jany, J.-L., Barbier, G., & Coton, E. (2014). Filamentous fungi and mycotoxins in cheese: A review. Comprehensive Reviews in Food Science and Food Safety, 13(4), 437–456. https://doi.org/10.1111/1541-4337.12069

Iqbal, S. Z., Jinap, S., Pirouz, A. A., & Ahmad Faizal, A. R. (2015). Aflatoxin M1 in milk and dairy products, occurrence and recent challenges: A review. Trends in Food Science & Technology, 46(1), 110–119. https://doi.org/10.1016/j.tifs.2015.08.005

Mahmudiono, T., Mazaheri, Y., Sadighara, P., Akbarlou, Z., Hoseinvandtabar, S., & Fakhri, Y. (2024). Prevalence and concentration of aflatoxin M1 and ochratoxin A in cheese: A global systematic review and meta-analysis and probabilistic risk assessment. Reviews on Environmental Health, 39(4), 801–840. https://doi.org/10.1515/reveh-2023-0069

Montagna, M. T., Santacroce, M. P., Spilotros, G., Napoli, C., Minervini, F., Papa, A., & Dragoni, I. (2004). Investigation of fungal contamination in sheep and goat cheeses in southern Italy. Mycopathologia, 158, 245–249. https://doi.org/10.1023/B:MYCO.0000041897.17673.2c

Pattono, D., Grosso, A., Stocco, P. P., Pazzi, M., & Zeppa, G. (2013). Survey of the presence of patulin and ochratoxin A in traditional semi-hard cheeses. Food Control, 33(1), 54–57. https://doi.org/10.1016/j.foodcont.2013.02.019

Pavicich, M. A., Compagnoni, S., Meerpoel, C., Raes, K., & De Saeger, S. (2024). Ochratoxin A and AFM1 in cheese and cheese substitutes: LC-MS/MS method validation, natural occurrence, and risk assessment. Toxins, 16(12), 547. https://doi.org/10.3390/toxins16120547

Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage (3rd ed.). Springer. https://link.springer.com/book/10.1007/978-0-387-92207-2

Rodríguez-Cañás, I., González-Jartín, J. M., Alvariño, R., Alfonso, A., Vieytes, M. R., & Botana, L. M. (2023). Detection of mycotoxins in cheese using an optimized analytical method based on a QuEChERS extraction and UHPLC-MS/MS quantification. Food Chemistry, 408, 135182. https://doi.org/10.1016/j.foodchem.2022.135182

Taniwaki, M. H., Hocking, A. D., Pitt, J. I., & Fleet, G. H. (2010). Growth and mycotoxin production by fungi in atmospheres containing 80% carbon dioxide and 20% oxygen. International Journal of Food Microbiology, 143(3), 218–225. https://doi.org/10.1016/j.ijfoodmicro.2010.08.030

Appendix A. Data and Statistical Clarifications

The original supplied summary included $t = 5.44$ for the soft-versus-hard count comparison and $\chi^2 = 7.28$ for mycotoxin detection. Neither statistic was retained as confirmed because raw CFU/g values, the exact definition of the "±" terms, and the contingency tables/hypotheses were not supplied. The reported count means and dispersions do not permit a transparent reproduction of the stated t statistic. The study should retain raw worksheets and rerun the final analysis before journal submission.

Issue	Required correction before submission
Count comparison	Provide individual CFU/g data, define SD or SEM, log-transform if required, report test assumptions, effect size, CI, and exact p value.
Fungal denominator	State whether one or multiple isolates were reported per sample; provide counts by cheese type and species.
AFM1 and OTA	Report ng/kg, LOD/LOQ, recovery, calibration, method validation, toxin co-occurrence, and positive/negative quality-control results.
Risk/compliance	Identify the applicable national or chosen reference standard and compare quantitative concentrations only.
Source attribution	Do not attribute AFM1 to a cheese isolate or OTA to a genus without source-tracing evidence.

Table A1. Essential data elements required to elevate the survey from screening evidence to a fully auditable food-safety study.

Appendix B. Reference Verification Note

The original reference list was standardized and reduced to sources for which a DOI, publisher page, official regulatory link, or book link was confirmed. The Word reference URLs are active hyperlinks. The companion Excel workbook contains the link list and data-integrity notes.