









RESEARCH ARTICLE

Stakeholder assessment for mycotoxin analysis: exploring the demand along the European food supply chain

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Received 19 September 2023 | Accepted 8 November 2023 | Published online 30 November 2023 |
Published in print 7 December 2023

Abstract

Mycotoxins are amongst the most prevalent food contaminants leading to serious health implications for humans and animals. Limiting exposure to them within the population remains a pressing food safety challenge. Prevention and timely detection are crucial for minimising mycotoxin contamination in food and feed. Therefore easy-to-use, rapid, eco-friendly and low-cost screening methods are increasingly implemented. Early-stage end-user engagement in the technological development process aids and guides the research towards increased societal impact. To investigate these end-user needs, the limitations of the currently used methods and the expectations towards a novel approach were mapped among stakeholders in a comprehensive survey. Stakeholders along the food supply chain (agricultural, food processing, retail, food safety and control, laboratories) were identified in five European countries and contacted. A total of 63 participants completed the survey, which was followed-up by an interview. The results of the survey revealed that different end-user groups have different priorities. Important limiting factors for agricultural, processing and retail stakeholders of the current methodologies include the complexity of sample preparation, high cost and time-to-results. Complementing the accredited laboratory tests with a pre-screening device would be especially interesting for agricultural producers (87.5% of the participants are interested) and food processors (80%), since there is an increasing demand for on-site detection of contamination. On the other hand, food control authorities and commercial laboratories indicated higher priority towards low quantification limits and multi-compound methods. The time to get the results was found to be more important than the testing cost (important aspect for 74.6 versus 66.7%). Overall, the findings of this study are critical input for end-user-targeted development of novel mycotoxin detection platforms.

Keywords

mycotoxin analysis – methodology – stakeholder assessment – requirements

1 Introduction

Mycotoxin contamination is one of the most significant safety issues along the food supply chain (Imade *et al.*, 2021; Kabak *et al.*, 2006; Streit *et al.*, 2013; Wagacha and Muthomi, 2008). Although mycotoxigenic fungi infestation is already a well-known risk (Milićević *et al.*, 2010), climate change is expected to lead to a significant shift in mycotoxin occurrence, resulting in serious challenges to current food safety and security measures (Chakraborty *et al.*, 2000; Medina *et al.*, 2017; Miraglia *et al.*, 2009). Mycotoxin contamination affects a wide range of foodstuffs including cereals (El-Sayed *et al.*, 2022; Femenias *et al.*, 2023; Pitt and Miller, 2017), dried fruits, nuts, and spices (Alshannaq and Yu, 2017). Recent research indicates that 60–80% of the investigated samples are contaminated with at least one mycotoxin worldwide (Eskola *et al.*, 2020). This leads to a severe human health burden and causes significant economic losses (Buzby, 2003; Cinar and Onbaşı, 2019; Gbashi, 2018; Mesterházy *et al.*, 2020; Popp *et al.*, 2014). Mycotoxin contamination poses a persistent food safety risk as, even at minimal consumption of mycotoxins (Agriopoulou *et al.*, 2020), chronic dietary exposure to these and other toxins needs to be considered (Nešić *et al.*, 2021).

To minimise the risk of chronic intake, maximum levels (MLs) of mycotoxin concentration in food are established internationally (López-García, 2022). In the European Union, the MLs are laid down in Regulation (European Commission) No 2023/915 for all foodstuffs (EC, 2023). Compliance with national and international regulations is the obligation of all food business operators. Low MLs are especially critical in certain food categories, such as baby food. The health and economic impact of mycotoxins leads to the ongoing development of different analytical techniques to detect them (Tittlemier *et al.*, 2022). These procedures can be divided into confirmatory and screening methods (Miklós *et al.*, 2020), which are (ideally) used in a complementary fashion. Screening methods are tools that ideally are suitable for non-experts, whereas lab-based confirmatory methods are required to confirm the presence of the mycotoxin. The latter type of technique is based on instrumental analysis like gas chromatography (GC) or liquid chromatography (LC) hyphenated with differ-

ent detection systems. Nowadays, LC coupled to tandem mass spectrometry (LC-MS/MS) is the gold standard for confirmatory multi-mycotoxin analysis (Malachová *et al.*, 2018). For screening of mycotoxins, immunoassays like lateral flow assays (LFAs) and enzyme-linked immunosorbent assays (ELISA) are mostly used (Li *et al.*, 2014; Zhou *et al.*, 2020). These immunoassays are the most frequently used analytical techniques resulting in a wide commercial availability of mycotoxin test kits often involving dedicated read-out systems (Nolan *et al.*, 2019). State-of-the-art assay kits require basic laboratory training and equipment, in addition to being consumable and less labour intense (Li *et al.*, 2014). Although cheaper than instrumental analysis, LFAs and ELISAs are still fairly expensive, preventing large-scale implementation outside the lab (Shah and Maghsoudlou, 2016). Another limiting factor of immunoassays is that they use antibodies (proteins) which can be sensitive to temperature changes and present cross-reactivity caused by substances from the matrix interfering with the antibody-antigen interactions (Laamanen and Veijalainen, 1992). Both methodologies have a significant environmental impact as confirmatory methods rely on organic solvents whereas screening techniques heavily rely on consumables (Lee and Ryu, 2015). Based on these limitations, there is a push on academia and industry to develop eco-friendly, portable, and simple methods for mycotoxin screening, which also enables on-site analysis.

Over the past decades, advanced technology platforms such as point-of-care (POC) and point-of-need (PON) have increasingly become embedded in our society and can range from pregnancy tests to diabetes monitoring devices and rapid mycotoxin test kits (Geballa-Koukoula *et al.*, 2023; Ross *et al.*, 2023). These platforms are developed in such a way that the user can perform the entire sample-to-result procedure by themselves. Therefore, it is extremely important to understand the end-user profile and include their needs early in the technology development process (Kasza *et al.*, 2022; Te Kulve and Rip, 2011). Since the 1990s, socio-technical frameworks such as the ethical, legal and societal implications (ELSI) program (Fisher, 2005) provided the foundation for more recent frameworks such as constructive technology assessment (CTA) (Rip and Robinson, 2019) and responsible research & innovation

(RRI) (Owen *et al.*, 2013). The CTA framework analyses the dynamics and arising permanent changes in a technology domain and creates socio-technical future scenarios. Such scenarios function as a starting point for interaction between stakeholders in workshops and for strategic implementation of the technology. By processing the input of all stakeholders, the societal impact of the technology development and contribution to RRI will be enhanced. This approach revolves around stakeholder engagement which is part of both CTA and RRI (Geballa-Koukoula *et al.*, 2023). In order to achieve end-user engagement and inclusiveness within (European) research projects, several tools from these frameworks could be used, such as stakeholder analysis, innovation value chain, multi-path map and stakeholder workshops (www.cta-toolbox.nl).

The field of mycotoxin analysis is constantly changing (Tittlemier *et al.*, 2022), as the stakeholder demands change (Adunphatcharaphon *et al.*, 2022). For instance, vibrational spectroscopy methods have been increasingly popular over the past decade due to their green approach and simple sample preparation procedures (Freitag *et al.*, 2022). One of the most demanded are near-infrared (NIR) (Shen *et al.*, 2022) and mid-infrared (MIR) spectroscopy (De Girolamo *et al.*, 2019; Femenias *et al.*, 2023), as well as NIR based hyperspectral imaging (NIR-HSI) (Femenias *et al.*, 2021, 2022a,b). A common pitfall during the development of novel (mycotoxin) detection methods is to not or only partially involve (potential) stakeholders during the research process. Despite its importance, involving different stakeholders is also challenging, as different sub-groups might have different expectations regarding the aim, requirements, and perception of analytical methods. For example, national reference laboratories usually perform confirmatory analysis, whereas the industry complies with regulations by simply screening for mycotoxins with rapid tests (Li *et al.*, 2014). Data obtained from the European Food Safety Authority (EFSA) confirms that national food safety authorities and reference laboratories predominately use LC-MS/MS and HPLC-FLD (Eskola *et al.*, 2020), whereas in industrial surveys ELISA are more often used, besides the beforementioned methods (Gruber-Dorninger *et al.*, 2019). The diversity of needs and mycotoxin testing routines supports that the clarification of the requirements of different stakeholders is needed.

Here, we map the mycotoxin-testing landscape along the food supply chain, and a stakeholder requirement survey is conducted to understand the needs and expect-

tations of the end-users of current, and innovative analytical solutions. The findings of this survey will provide guidance for the development of novel mycotoxin screening methods, stimulating uptake by academic, industrial and official laboratories.

2 Materials and methods

Identifying relevant stakeholders

The concept of the stakeholder requirements survey was to include all actors of the food chain for which mycotoxin testing might be relevant. The importance of monitoring mycotoxin contamination in primary production is unchallengeable (Schatzmayr and Streit, 2013), and therefore participants from the agricultural sector represent a key player for the survey. This sector included not only agricultural commodity producers but also stakeholders involved in storing, handling, and re-selling primary food products and feed as well. The quality assurance practices of food processors and retailers – dealing with products with possible mycotoxin contamination – includes the continuous monitoring of different mycotoxins, both with rapid on-site tests and confirmatory methods. The participating food business operators included small and medium-sized enterprises (SMEs) and multinational food producers, wholesalers, and distributors as well. Several of the contacted stakeholders have more than one focus area, being involved not only in food production but also in exporting their products through countries and continents. Service and governmental laboratories (with border inspection points) play a crucial role in maintaining food safety along the food chain, with high testing frequency in the area of mycotoxins. The targeted participants of the stakeholder requirements survey thus were selected accordingly. 206 stakeholders from these key groups along the food supply chain (agricultural, food processing, retail, food safety and control, laboratories) were identified in five European countries (Austria, Germany, Hungary, the Netherlands and Norway) and contacted. As a result of mapping all the stages of the food supply chain, we managed to gather an extensive database, considering stakeholders needs and expectations in the field of mycotoxin testing practices. Even with the comprehensive sampling of stakeholders, we have identified several knowledge gaps, regarding the exact testing procedures, especially if the testing is not done directly by the stakeholder, but by an external laboratory.

Stakeholder requirements survey

The survey questionnaire and the follow-up interview protocol were composed based on a series of online meetings by a multi-disciplinary team, including experts from the field of mycotoxin detection, technology development, stakeholder engagement and end-user studies. Besides the selected group of developers, actual stakeholders in the field of mycotoxin testing were also involved in the process.

The survey aimed to gather data about the currently used mycotoxin testing methods and the advantages and disadvantages perceived by the stakeholders. The stakeholder survey included two parts. An online questionnaire – including 13 open- and 8 close-ended questions – was performed, followed by a structured interview, allowing to collect individual opinions and expectations as well. The stakeholder survey addressed three sub-topics: (1) general information about the stakeholder mycotoxin testing strategy, (2) mapping the strengths and weaknesses of the currently available methods, and (3) the preferred directions of innovations. The online survey was implemented via Microsoft Forms (Microsoft, Redmond, WA, USA). During the data collection period (between May 2021 and April 2022), 63 completely filled out surveys were collected for the online questionnaire.

80.9% of the completed surveys (51/63) were successfully followed up with an interview, conducted via telephone, online conference platforms or in person. The interviews took place at pre-arranged times, between June 2021 and May 2022, based on a structured interview guide (SM2). The interviews were recorded and transcribed based on the signed consent of the participant. During the interviews, participants briefly introduced the organisation they represented, the relevant food safety risks associated with mycotoxin contamination and the mycotoxin testing methods used by their organisation. They also shared their ideas about what possible developments could support their daily work to detect mycotoxins in food. The stakeholder survey questionnaire and the follow-up interview protocol are available in its totality in the Supplementary Material S1 and S2.

Data analysis

The collected data was processed and analysed in Microsoft Office 2019 and IBM SPSS Statistics 22.0 (Armonk, NY, USA) and supported by visual presentation of the data. The survey data was analysed separately for the different stakeholder groups along the food supply chain, defining the specific requirements of agri-

culture, food processing, food retail, laboratories, and food control organisations. The priorities of the different sectors were also formulated, outlining the possible directions of future technology development, based on the targeted end-user group.

3 Results and discussion

Characterisation of the participants

In the framework of the stakeholder requirements survey, besides mapping the currently used mycotoxin testing methods, the user expectations towards novel innovations were also identified among an extensive range of end-users, covering different stages of the food chain: agriculture, food industry, food trade and retail, food safety authorities (with border inspection points) and service laboratories in different countries (Figure 1).

The focus areas of the participating organisations are also summarised in Figure 1. Most participants occupy more than one focus area, resulting in a total of more than 100% in the table. The participating organisations covered a wide range of stakeholders not only according to their main focus but also according to the volume of mycotoxin tests performed annually. Their sample throughput differs from <50 samples per year (30.2%) to thousands of samples per year (33.3%), as illustrated in Figure 1.

To understand the current processes and daily routines in the detection of mycotoxin contamination, the location of mycotoxin testing was also reviewed within the stakeholder groups. As summarised in Figure 2 it was found that mycotoxin measurements are predominantly conducted in-house, especially in the sectors with responsibility for food control and laboratory analytics. However, food processors and retail companies often outsource the analysis to external accredited laboratories, while the agricultural sector shows varied approaches. 6.3% of the total respondents do not conduct any mycotoxin tests currently (13.3% in the agriculture and food industry (subgroup A), and 4.8% in food control sector and laboratories (subgroup B), but intended to broaden their testing routine with mycotoxin analysis in the near future.

Currently used methods for mycotoxin testing

Concerning the testing methods that are currently used, LC-MS/MS, ELISA and LC-UV/FLD are the most common in the everyday routine of mycotoxin testing (Figure 3). Besides the conventional methods, several rapid tests were also mentioned, such as the Biochip Myco

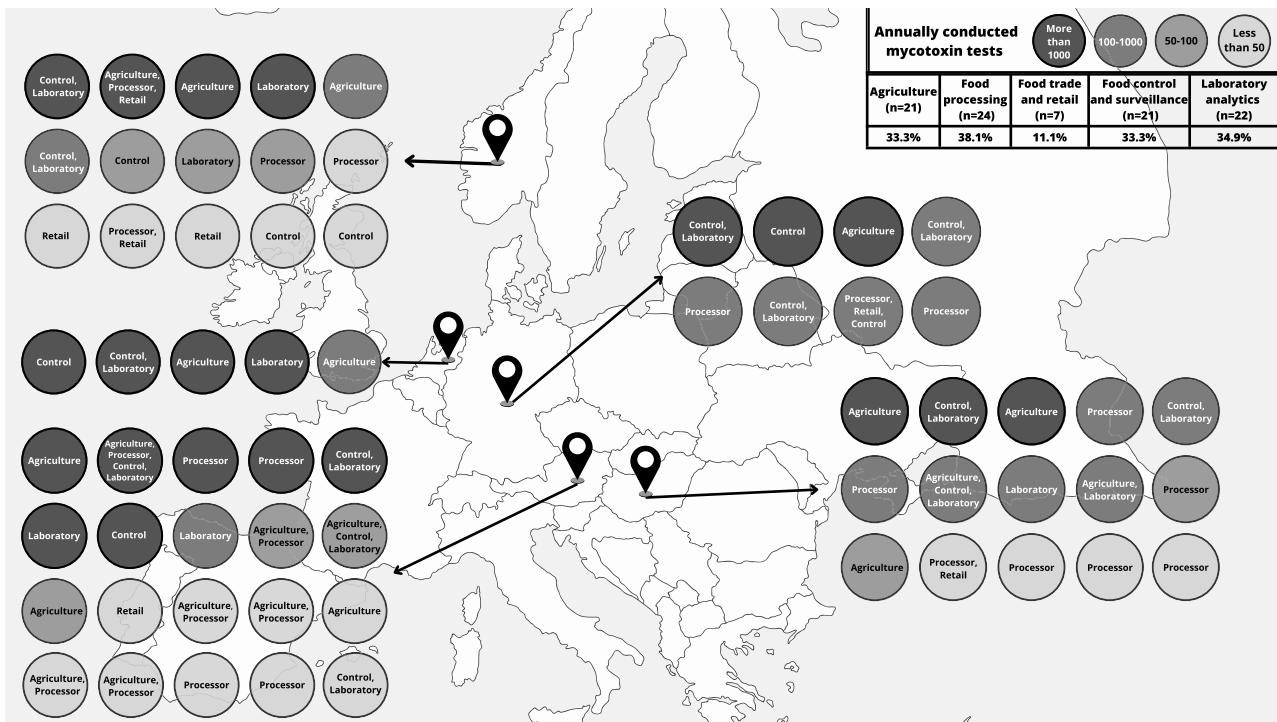


FIGURE 1 Map of the participating stakeholders per country (summarising the relevant stages of the food supply chain and the volume of annual mycotoxin tests).

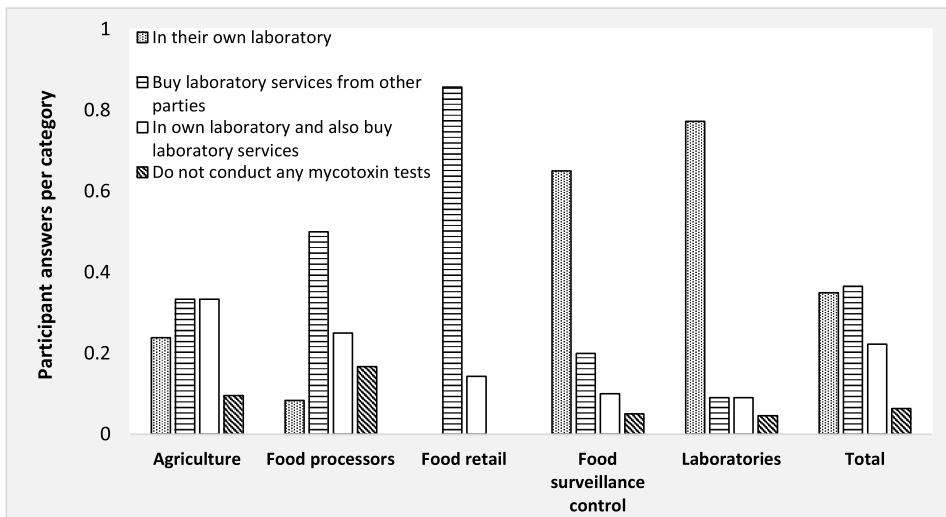


FIGURE 2 The location of mycotoxin testing in different sectors of the food supply chain ('How does your organisation conduct tests on mycotoxin contamination?').

7 Array which is a chemiluminescent immunoassay method that could quantitatively detect seven mycotoxins simultaneously (Radox Food Diagnostics, Crumlin, UK). Recently, the device was evaluated across multiple laboratories during a proficiency testing round and was found to be fit-for-purpose for the use in the Association of American Feed Control Officials (AAFCO) control programs for mycotoxins (Freitas *et al.*, 2019; Sibanda *et al.*, 2022). Furthermore, the ROSA (Rapid

One Step Assay) for deoxynivalenol (DONQ2) and aflatoxin (AFQ) was mentioned by the participants of the survey. These are lateral flow immunoassay test kits that are available for the quantitative analysis of seven individual mycotoxins in a multitude of commodities (Charm Sciences, Lawrence, MA, USA; Salter *et al.*, 2019). These kits require an additional incubator and reader system provided by the manufacturer to perform the tests. Lastly, the RIDASCREEN® kits (R-Biopharm AG,

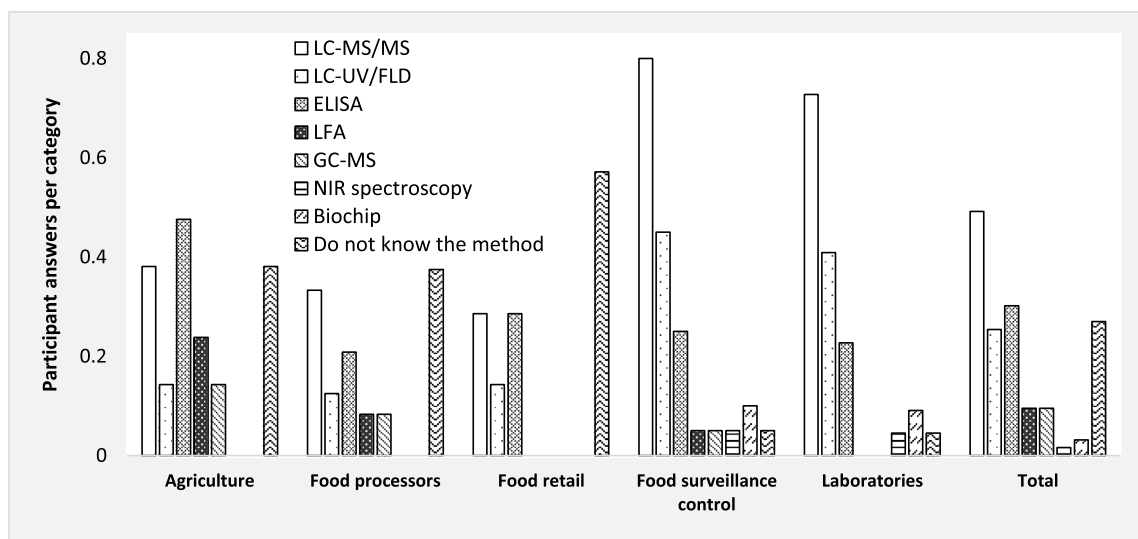


FIGURE 3 Current methods for mycotoxin detection ('What type of laboratory test does your organisation apply for mycotoxin detection?'). LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry; LC-UV/FLD = liquid chromatography with ultraviolet or fluorescence detection; ELISA = enzyme-linked immunosorbent assays; LFA = lateral flow assay; GC-MS = gas chromatography coupled to mass spectrometry, NIR = near-infrared.

Darmstadt, Germany) for mycotoxins were employed, which are ELISA tests. Moreover, the usage of the above-mentioned rapid tests by the survey participants was in line with the described trends for mycotoxin screening (Nolan *et al.*, 2019).

It is important to notice that 27.0% of the participants did not know which method was used for mycotoxin detection; this number is especially high (56.5%) in the case of stakeholders that outsource mycotoxin testing.

The complexity of the mycotoxin testing framework is well demonstrated by the fact that although monitoring efforts – as part of the daily routine – are performed by an in-house laboratory or through rapid tests, the customer demands or quality assurance systems require confirmation by an accredited external laboratory. Besides, stakeholders with responsibilities in the field of food control and laboratory analytics all use some type of instrumental analytical methods for mycotoxin detection with no external laboratory involved; primarily LC-MS/MS, but also LC-UV/FLD was reported. This finding is linked to the fact that confirmatory methods have to comply with certain regulatory requirements for mycotoxin identification on a molecular level (EC, 2002). Service laboratories cover a wide range of methods, including ELISA, and sporadically Biochip and near-infrared (NIR) spectroscopy as well. The implementation of the latter techniques is surprising, as NIR spectroscopy without doubt, has its advantages, but is not yet well-established for mycotoxin analysis (Freitag *et al.*, 2022). Immunoassays have been referred to as

the dominantly used mycotoxin testing methodology (Nolan *et al.*, 2019). Our findings (see Figure 3) underline that statement partly as agricultural producers reported using primarily rapid screening methods, such as ELISAs and lateral flow devices, after which positively screened samples are sent for confirmatory analysis by e.g. LC-MS/MS or LC-UV/FLD. This is in good accordance with the scientific literature on mycotoxin surveys, where it has been shown that industrial stakeholders rely on a higher proportion of immunoassays but also instrumental analysis (Gruber-Dorninger *et al.*, 2019), whereas national reference laboratories have a clear preference for LC-MS/MS or HPLC-FLD (Eskola *et al.*, 2020).

Limiting factors of the currently used methods

According to the participants, current methods can be characterised by high unit cost (total analysis cost), accuracy/reliability problems, and the long turnaround time (Figure 4). For lab-based methods, the cost and time parameters are the limiting factors, besides the infrastructure and qualified staff needed for the measurements. Insufficient reliability and the acceptance of business partners are reported to be more problematic for those participants who use screening methods. In addition, for organisations where mycotoxin testing is performed on-site, sample preparation was the most frequently mentioned bottleneck during the follow-up interviews. Lengthy homogenisation, grinding, and sample purification are particularly significant problems in this respect, especially since the majority of participants do not use multi-toxin methods, so testing a sample for

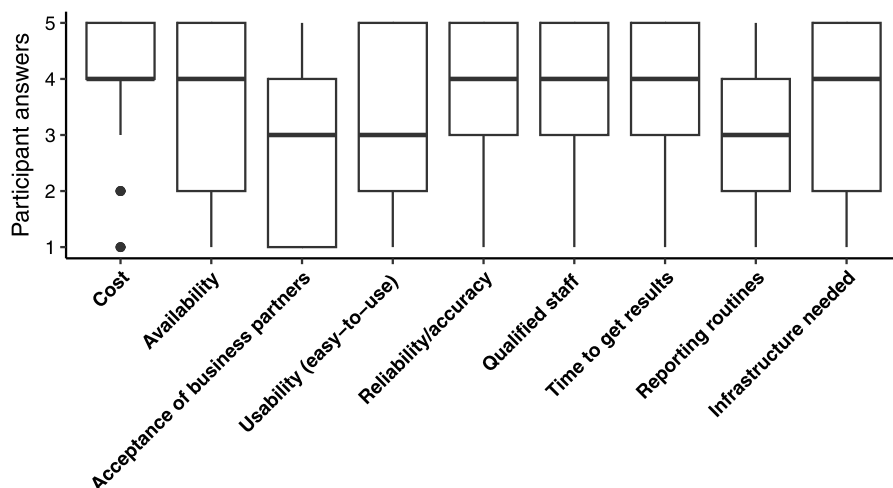


FIGURE 4 Limiting factors of the current methodology for all stakeholder groups ('What are the most important limitation factors of using the current technology more frequently for mycotoxin testing?'). 1 – not important, 5 – very important.

more than one toxin requires separate sample preparation for each case.

Satisfaction with the currently used methods

Despite the limiting factors, based on the findings of the stakeholder survey, the majority of the participants are satisfied with the methods they use. The follow-up interviews, however, highlighted that there are several bottlenecks, that with improvement, would be beneficial for the companies. This included less complicated, cheaper and especially more rapid testing, which would lead to a more efficient organisation of their production and better service towards customers and suppliers. The average satisfaction with the current methods of the participating organisations was 7.1 out of 10, ranging from 6.5 (agriculture) to 7.7 (official and service laboratories). This was considerably lower in the case of stakeholders that partially or entirely outsource mycotoxin tests to an external laboratory (the average is 6.7 if the testing is outsourced, 7.8 if the testing is done in the own laboratory), underlining the importance of rapid results and low costs.

Stakeholder requirements towards a novel technology

The stakeholder survey identified the prioritised aspects and attributes of a preferred novel method, which represents differences for the particular sectors of the food supply chain (Figure 5). Based on the results, two subgroups of stakeholders were identified, having similar priorities and mycotoxin testing practices: farmers and food processors in subgroup A and service laboratories and food control authorities in subgroup B.

For the representatives of the agricultural sector, the most important parameter is the low cost per sample

(important for 81% of the participants). Rapid results are also prioritised (76.2%) as it would foster efficient on-site testing and promote quick decision-making. Not having to rely on complicated sample preparation (39.5% in subgroup A versus 17.9% in subgroup B), and requiring qualified staff for the operation are also more important for these stakeholders than for others (34.2% in subgroup A versus 7.1% in subgroup B). The portability of the testing device is relatively important in this end-user group (21.1% in subgroup A versus 7.1% in subgroup B). Food processing companies in the survey were mostly small-scale testers, conducting less than 50 mycotoxin tests annually (Figure 1). Time-effectiveness and rapid results are especially important for these stakeholders. We found that they enquire laboratory services from other parties – 47.8% of them outsource the entire process, and 26.1% partially outsource their mycotoxin testing process – which usually results in a long time (often up to two-three weeks) until the desired result is obtained.

The expectation of the food trade and retail sector about mycotoxin testing is rather specific, as they often require a certificate confirming the compliance of the product to be presented by the suppliers. Based on the survey data, 85.7% of the retailers outsource mycotoxin testing, resulting in less information about the currently applied method and also about the exact requirements towards a novel method. However, rapid results and low unit cost are important for most of the participants.

The representatives of the official food control sector (such as national authorities, border control services) often operate their own laboratory for mycotoxin testing (63.2%). They reported a high annual sample throughput: 47.7% of the participants said that their

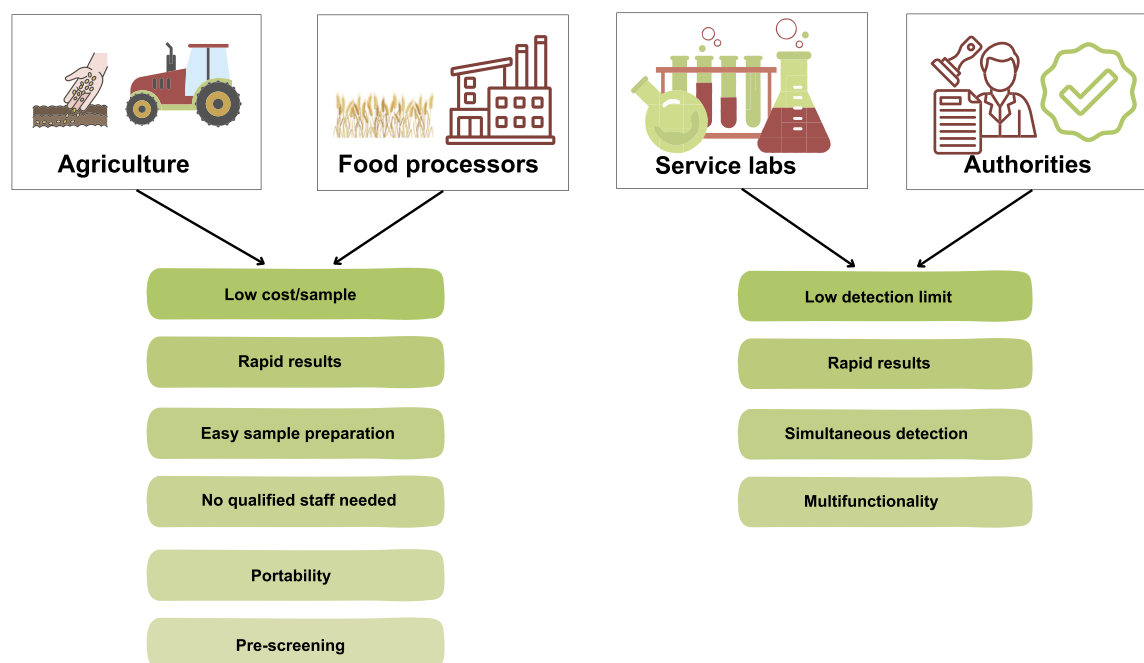


FIGURE 5 The priorities of the stakeholder requirements in the different sectors of the food supply chain.

organisation performs more than 1000 analyses per year (Figure 1). Most of them rely on confirmatory methods, such as LC-MS/MS (78.9%) and LC-UV/FLD (42.1%) (Figure 3). This is linked to the fact that a low limit of quantification (LOQ) is highly important in this sector (67.9% in subgroup B versus 44.7% in subgroup A). Optimising on low cost per analysis is less prevalent (46.4% in subgroup B versus 76.3% in subgroup A) than in other sectors and in good accordance with the literature (Eskola *et al.*, 2020). It must be noted that data security is more important (25.0% in subgroup B versus 15.8% in subgroup A) for them than for other stakeholders. The requirements of the border inspection points were analysed in parallel with food control authorities, as food safety measurements on the border are usually the responsibility of the national authorities. The survey results confirmed that testing does not take place at the border points with rapid tests, but the samples are taken to accredited laboratories – generally maintained by governmental institutions – and tested with high-performance confirmatory methods. Currently used methods in this sector are LC-MS/MS and LC-UV/FLD. However, border inspection points reported that they would be interested in a pre-screening tool, to identify possibly contaminated samples. The survey participants that offer laboratory services for commercial purposes defined similar requirements as the representatives of the food control sector. Multi-functionality of the device is crucial in this stakeholder group (57.1% in subgroup B versus 39.5%

in subgroup A) and low LOQ is more important than for other industry users. Qualified staff and sample preparation procedures (9.1% versus 28.6% in general) are less problematic for them, as they usually have the necessary experts and laboratory infrastructure in place.

Complementing the accredited laboratory tests with a pre-screening device would be especially interesting for agricultural producers (87.5% of the participants are interested) and food processors (80%), as there is an increasing demand for on-site detection of contamination (Choi *et al.*, 2017; Umapathi *et al.*, 2022). In parallel, these sectors are less satisfied with their currently used methods (6.6 versus 7.5 in subgroup B).

Forecasting the performance characteristics of a novel method is a crucial part of technology development. However, mapping the stakeholder requirements in this field poses a challenge, as most of them – especially the non-laboratory experts – are not able to describe their exact needs from a laboratory method. The most critical point to many of them is to receive a test result that meets the stipulated European maximum limits. Based on the beforementioned differences between the two subgroups with regards to the LOQ it can be assumed that for subgroup B the actual performance criteria of the used method is more important than for subgroup A. The latter group could be satisfied with a method simply reporting the compliance of the sample under investigation to a certain method. Most of the respondents could not quantify the expected LOQ, concentration range and measurement

uncertainty. Most of the survey participants did not provide exact numbers, only emphasising the importance of meeting the 'requirements of food safety authorities'. Therefore, the exact performance characteristics can be indirectly set based on the stakeholder input. For the European market, the LOQ of the novel methods for mycotoxin analysis is set by the performance criteria defined by the European Commission (EC, 2006, 2023). According to this, the limit of quantification shall be at least $\leq 0.5 \times$ maximum level (ML), except for certain strict maximum levels, in which cases the LOQ is equal to the ML. In case the ML applies to a sum of toxins, then the LOQ of the individual toxins shall be $\leq 0.5 \times$ ML/n, with n being the number of toxins included in the ML definition (WUR – EURL).

The cost implications of mycotoxin analysis turned out to be a complex issue. While the study did not ask for specific cost estimation for the certain methods, we asked the respondents to evaluate the importance of this element compared to other criteria. Although cost was reported to be the most important limiting factor of using the current methods more frequently (Figure 4), this is not the only critical aspect of the installation of a novel method (being important for 66.7% of the participants). The time to get the results was found to be more important than the testing cost (important aspect for 74.6 versus 66.7%). The follow-up interviews revealed the reasons behind that, as waiting for the laboratory results often delivers significant indirect costs, such as expanded storage cost and pauses in the supply chain which greatly exceed the cost of the quality assurance measurements. It is more important to have a reliable method which is approved by the business partners and authorities, with optimal performance characteristics than to cut down the unit cost by a few percentages. One quarter of the participants also mentioned that they would consider changing the current method even without a cost reduction in favour of a solution with a shorter testing time and ideal performance characteristics of the methodology.

4 Conclusions

The stakeholder survey results confirmed that all actors in the food chain are aware of their role and responsibility in monitoring mycotoxin contamination. Understanding the bottlenecks and limiting factors of a currently used method should be the first step in a novel technology development process. In organisations where testing is done in-house, the main limitation is

the complicated sample preparation and purification. In organisations where testing is performed by an external accredited laboratory, time and high-throughput factors are the most common problem. Although different sectors have different demands regarding a novel method for mycotoxin testing, time-effectiveness, rapid and approved results are the most important factors in general. As for replacing or complementing the current methods with a novel technology, cost reduction proved to be less important than the time to get the results.

The different stakeholder groups formulated their expectations and the most important parameters of mycotoxin testing methods. Economic aspects, such as low cost/sample are crucial for agriculture, food processors and the retail sector. The representatives from the agriculture sector highlighted the importance of the portability of the device, enabling monitoring in the field. The issues related to complex sample preparation and need for qualified staff are important for the agriculture and food processors, but to a lesser extent for authorities and laboratories, as they already have the necessary infrastructure and know-how available. Low LOQ and multifunctionality – enabling to target different analytes – are extremely important for food control authorities, official and service laboratories conducting a high number of measurements daily. Since the representatives of agriculture, food processors and the retail sector reported lower satisfaction with their current method, they might be more willing to change or complement their current testing routine. In parallel, agriculture, food processors and border inspection points would be really interested in a novel screening device.

Mapping the status and bottlenecks of the currently used mycotoxin testing methods outlines the direction of future technology development processes. As the daily routine analysis of mycotoxins shows a very diverse picture, a novel mycotoxin testing method and device would rather complement the current methods or increase the number of mycotoxin tests performed than entirely replace the widespread current methods. It is important to mention that several stakeholders, and especially those that outsource mycotoxin testing are not able to recall LOQ, concentration range and measurement uncertainty, and identified only the importance of meeting the 'requirements of food safety authorities'. As much as it might be concerning, it also indicates a need for rapid but reliable mycotoxin screening methods, which enable various stakeholders along the food supply chain to make quick decisions on the safety of the commodity under investigation. Multi-

toxin methods could be also an important research direction in mycotoxin detection, no matter what the technology is behind.

Supplementary material

Supplementary material is available online at: <https://doi.org/10.6084/m9.figshare.24560146>

Materials S1. Questionnaire of the stakeholder survey.
Materials S2. Protocol of the follow-up interview.

Acknowledgements and funding

The work presented in this publication was conducted as part of the PHOTONFOOD project, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101016444 and is part of the Photonics21 Photonics Public Private Partnership <https://www.photonics21.org>.

Authors' contribution

Conceptualisation, GK and JS; methodology, GK, JS, RK, BM and GS; formal analysis, PF, MH, AFL, AJB, FSR, MS, SF, MV, EC; writing – original draft preparation, GK, EC, VM; writing – review and editing, PF, AFL, AJB, SF, MV, FSR, ES, GS, and GK; visualisation, VM, EC and SF; supervision, BM, JS, GS, FSR, RK and GK; project administration, MS; funding acquisition, VM, MS. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets generated during the current study will be available in the Zenodo repository.

References

- Adunphatcharaphon, S., Elliott, C.T., Sooksimuang, T., Charlermroj, R., Petchkongkaew, A. and Karoonuthaisiri, N., 2022. The evolution of multiplex detection of mycotoxins using immunoassay platform technologies. *Journal of Hazardous Materials* 432: 128706. <https://doi.org/10.1016/j.jhazmat.2022.128706>
- Agriopoulou, S., Stamatelopoulou, E. and Varzakas, T., 2020. Advances in analysis and detection of major mycotoxins in foods. *Foods* 9: 518. <https://doi.org/10.3390/foods9040518>
- Alshannaq, A. and Yu, J.H., 2017. Occurrence, toxicity, and analysis of major mycotoxins in food. *International Journal of Environmental Research and Public Health* 14(6): 632. <https://doi.org/10.3390/ijerph14060632>
- Buzby, J.C., 2003. International trade and food safety: economic theory and case studies. United States Department of Agriculture. Economic Research Service, Washington, DC, USA.
- Chakraborty, S., Tiedemann, A.V. and Teng, P.S., 2000. Climate change: potential impact on plant diseases. *Environmental Pollution* 108: 317-326. [https://doi.org/10.1016/S0269-7491\(99\)00210-9](https://doi.org/10.1016/S0269-7491(99)00210-9)
- Charm Sciences, nd. ROSA Lateral Flow Mycotoxin Strips. Available at: <https://www.charm.com/products/test-and-kits/mycotoxin-tests/rosa-lateral-flow/>
- Choi, J., Seong, T.W., Jeun, M. and Lee, K.H., 2017. Field-effect biosensors for on-site detection: recent advances and promising targets. *Advanced Healthcare Materials* 6: 1700796. <https://doi.org/10.1002/adhm.201700796>
- Cinar, A. and Onbaşı, E., 2019. Mycotoxins: the hidden danger in foods. In: Marc, R.A. (ed.) *Mycotoxins and food safety – recent advances*. Intech Open, Rijeka, Croatia. <https://doi.org/10.5772/intechopen.89001>
- De Girolamo, A., Cervellieri, S., Cortese, M., Porricelli, A.C.R., Pascale, M., Longobardi, F., Holst, C., Ciaccheri, L. and Lipopolis, V., 2019. Fourier transform near-infrared and mid-infrared spectroscopy as efficient tools for rapid screening of deoxynivalenol contamination in wheat bran. *Journal of the Science of Food and Agriculture* 99: 1946-1953. <https://doi.org/10.1002/jsfa.9392>
- El-Sayed, R.A., Jebur, A.B., Kang, W., El-Esawi, M.A. and El-Demerdash, F.M., 2022. An overview on the major mycotoxins in food products: characteristics, toxicity, and analysis. *Journal of Future Foods* 2: 91-102. <https://doi.org/10.1016/j.jfutfo.2022.03.002>
- Eskola, M., Kos, G., Elliott, C.T., Hajšlová, J., Mayar, S. and Krška, R., 2020. Worldwide contamination of food-crops with mycotoxins: validity of the widely cited 'FAO estimate' of 25%. *Critical Reviews in Food Science and Nutri-*

- tion 60: 2773-2789. <https://doi.org/10.1080/10408398.2019.1658570>
- European Commission (EC), 2002. 2002/657/EC: Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. Official Journal of the European Union L 221: 8-36.
- European Commission (EC), 2006. Commission Regulation (EC) No 401/2006 of 23 February 2006 laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in foodstuffs. Official Journal of the European Union 70: 12-34.
- European Commission (EC), 2023. Commission Regulation (EC) No 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Official Journal of the European Union 119: 103-157.
- Femenias, A., Bainotti, M.B., Gatius, F., Ramos, A.J. and Marín, S., 2021. Standardization of near infrared hyperspectral imaging for wheat single kernel sorting according to deoxynivalenol level. *Food Research International* 139: 109925. <https://doi.org/10.1016/j.foodres.2020.109925>
- Femenias, A., Llorens-Serentill, E., Ramos, A.J., Sanchis, V. and Marín, S., 2022a. Near-infrared hyperspectral imaging evaluation of fusarium damage and DON in single wheat kernels. *Food Control* 142: 109239. <https://doi.org/10.1016/j.foodcont.2022.109239>
- Femenias, A., Gatius, F., Ramos, A.J., Teixido-Orries, I. and Marín, S., 2022b. Hyperspectral imaging for the classification of individual cereal kernels according to fungal and mycotoxins contamination: a review. *Food Research International* 155: 111102. <https://doi.org/10.1016/j.foodres.2022.111102>
- Femenias, A., Fomina, P., Tafintseva, V., Freitag, S., Shapaval, V., Sulyok, M., Zimmermann, B., Marín, S., Krska, R., Kohler, A. and Mizaikoff, B., 2023. Optimizing extraction solvents for deoxynivalenol analysis in maize via infrared attenuated total reflection spectroscopy and chemometric methods. *Analytical Methods* 15: 36-47. <https://doi.org/10.1039/D2AY00995A>
- Fisher, E., 2005. Lessons learned from the Ethical, Legal and Social Implications program (ELSI): planning societal implications research for the National Nanotechnology Program. *Technology in Society* 27: 321-328. <https://doi.org/10.1016/j.techsoc.2005.04.006>
- Freitag, S., Sulyok, M., Logan, N., Elliott, C.T. and Krska, R., 2022. The potential and applicability of infrared spectroscopic methods for the rapid screening and routine analysis of mycotoxins in food crops. *Comprehensive Reviews in Food Science and Food Safety* 21: 5199-5224. <https://doi.org/10.1111/1541-4337.13054>
- Freitas, A., Barros, S., Brites, C., Barbosa, J. and Silva, A.S., 2019. Validation of a biochip chemiluminescent immunoassay for multi-mycotoxins screening in maize (*Zea mays* L.). *Food Analytical Methods* 12: 2675-2684. <https://doi.org/10.1007/s12161-019-01625-1>
- Gbashi, S., Madala, N.E., De Saeger, S., De Boevre, M., Adekoya, I., Adebo, O.A. and Njobeh, P.B., 2018. The socio-economic impact of mycotoxin contamination in Africa. In: Njobeh, P.B. (ed.) *Fungi and mycotoxins – their occurrence, impact on health and the economy as well as pre-and postharvest management strategies*. InTech Open, Rijeka, Croatia. <https://doi.org/10.5772/intechopen.79328>
- Geballa-Koukoula, A., Ross, G.M.S., Bosman, A.J., Zhao, Y., Zhou, H., Nielen, M.W.F., Rafferty, K. and Salentijn, G.I.J., 2023. Best practices and current implementation of emerging smartphone-based (bio) sensors – part 2: development, validation, and social impact. *Trends in Analytical Chemistry* 161: 116986. <https://doi.org/10.1016/j.trac.2023.116986>
- Gruber-Dorninger, C., Jenkins, T. and Schatzmayr, G., 2019. Global mycotoxin occurrence in feed: a ten-year survey. *Toxins* 11: 375. <https://doi.org/10.3390/toxins11070375>
- Imade, F., Ankwasa, E.M., Geng, H., Ullah, S., Ahmad, T., Wang, G., Zhang, C., Dada, O., Xing, F., Zheng, Y. and Liu, Y., 2021. Updates on food and feed mycotoxin contamination and safety in Africa with special reference to Nigeria. *Mycology* 12: 245-260. <https://doi.org/10.1080/21501203.2021.1941371>
- Kabak, B., Dobson, A.D. and Var, I.I.L., 2006. Strategies to prevent mycotoxin contamination of food and animal feed: a review. *Critical Reviews in Food Science and Nutrition* 46: 593-619. <https://doi.org/10.1080/10408390500436185>
- Kasza, G., Csenki, E., Szakos, D. and Izsó, T., 2022. The evolution of food safety risk communication: models and trends in the past and the future. *Food Control* 138: 109025. <https://doi.org/10.1016/j.foodcont.2022.109025>
- Laamanen, I. and Veijalainen, P., 1992. Factors affecting the results of T-2 mycotoxin ELISA assay. *Food Additives and Contaminants* 9: 337-343. <https://doi.org/10.1080/02652039209374079>
- Lee, H.J. and Ryu, D., 2015. Advances in mycotoxin research: public health perspectives. *Journal of Food Science* 80(12): 2970-2983. <https://doi.org/10.1111/1750-3841.13156>
- Li, W., Powers, S. and Dai, S.Y., 2014. Using commercial immunoassay kits for mycotoxins: 'joys and sorrows'? *World Mycotoxin Journal* 7: 417-430. <https://doi.org/10.3920/WMJ2014.1715>
- López-García, R., 2022. Mycotoxin management: an international challenge. In: Martinović, A., Oh, S. and Lelieveld, H. (eds.) *Ensuring global food safety*. Academic Press, London, UK, pp. 213-219. <https://doi.org/10.1016/B978-0-12-816011-4.00024-0>

- Malachová, A., Stránská, M., Václavíková, M., Elliott, C.T., Black, C., Meneely, J., Hajslová, J., Ezekiel, C.N., Schuhmacher, R. and Krska, R., 2018. Advanced LC–MS-based methods to study the co-occurrence and metabolization of multiple mycotoxins in cereals and cereal-based food. *Analytical and Bioanalytical Chemistry* 410: 801-825. <https://doi.org/10.1007/s00216-017-0750-7>
- Medina, A., Akbar, A., Baazeem, A., Rodriguez, A. and Magan, N., 2017. Climate change, food security and mycotoxins: do we know enough? *Fungal Biology Reviews* 31: 143-154. <https://doi.org/10.1016/j.fbr.2017.04.002>
- Mesterházy, Á., Oláh, J. and Popp, J., 2020. Losses in the grain supply chain: causes and solutions. *Sustainability* 12: 2342. <https://doi.org/10.3390/su12062342>
- Miklós, G., Angeli, C., Ambrus, Á., Nagy, A., Kardos, V., Zentai, A., Kerekes, K., Farkas, Z., Józwiak, Á. and Bartók, T., 2020. Detection of aflatoxins in different matrices and food-chain positions. *Frontiers in Microbiology* 11: 1916. <https://doi.org/10.3389/fmicb.2020.01916>
- Miličević, D.R., Škrinjar, M. and Baltić, T., 2010. Real and perceived risks for mycotoxin contamination in foods and feeds: challenges for food safety control. *Toxins* 2: 572-592. <https://doi.org/10.3390/toxins2040572>
- Miraglia, M., Marvin, H.J.P., Kleter, G.A., Battilani, P., Brera, C., Coni, E., Cubadda, F., Croci, L., De Santis, B., Dekkers, S., Filippi, L., Hutjes, R.W.A., Noordam, M.Y., Pisante, M., Piva, G., Prandini, A., Toti, L., Van den Born, G.J. and Verspermann, A., 2009. Climate change and food safety: an emerging issue with special focus on Europe. *Food and Chemical Toxicology* 47: 1009-1021. <https://doi.org/10.1016/j.fct.2009.02.005>
- Nešić, K., Habschied, K. and Mastanjević, K., 2021. Possibilities for the biological control of mycotoxins in food and feed. *Toxins* 13: 198. <https://doi.org/10.3390/toxins13030198>
- Nolan, P., Auer, S., Spehar, A., Elliott, C.T. and Campbell, K., 2019. Current trends in rapid tests for mycotoxins. *Food Additives and Contaminants Part A* 36: 800-814. <https://doi.org/10.1080/19440049.2019.1595171>
- Owen, R., Stilgoe, J., Macnaghten, P., Gorman, M., Fisher, E. and Guston, D., 2013. A framework for responsible innovation. In: Owen, R., Bessant, J. and Heintz, M. (eds.) *Responsible innovation: managing the responsible emergence of science and innovation in society*. Wiley, Hoboken, NJ, USA, pp. 27-50. <https://doi.org/10.1002/9781118551424.ch2>
- Pitt, J.I. and Miller, J.D., 2017. A concise history of mycotoxin research. *Journal of Agricultural and Food Chemistry* 65: 7021-7033. <https://doi.org/10.1021/acs.jafc.6b04494>
- Popp, J., Lakner, Z., Harangi-Rákos, M. and Fari, M., 2014. The effect of bioenergy expansion: food, energy, and environment. *Renewable and Sustainable Energy Reviews* 32: 559-578. <https://doi.org/10.1016/j.rser.2014.01.056>
- Randox Food Diagnostics, nd. Myco 7 Array. Available at: <https://www.randoxfood.com/biochip/myco-7-array/>
- Rip, A. and Robinson, D.K., 2019. Constructive technology assessment and the methodology of insertion. In: *Nanotechnology and its governance*. Routledge, London, UK, pp. 128-144. https://doi.org/10.1007/978-94-007-7844-3_3
- Ross, G.M.S., Zhao, Y., Bosman, A.J., Geballa-Koukoula, A., Zhou, H., Elliott, C.T., Nielen, M.W.F., Rafferty, K. and Salentijn, G.I.J., 2023. Best practices and current implementation of emerging smartphone-based (bio) sensors – part I: data handling and ethics. *Trends in Analytical Chemistry* 158: 116863. <https://doi.org/10.1016/j.trac.2022.116863>
- Salter, R., Douglas, D., Tess, M., Markovskiy, B., Saul, S.J. and Brandt, A., 2006. Interlaboratory study of the Charm ROSA Safe Level Aflatoxin MI Quantitative lateral flow test for raw bovine milk. *Journal of AOAC International* 89: 1327-1334. <https://doi.org/10.1093/jaoac/89.5.1327>
- Schatzmayer, G. and Streit, E., 2013. Global occurrence of mycotoxins in the food and feed chain: facts and figures. *World Mycotoxin Journal* 6: 213-222. <https://doi.org/10.3920/WMJ2013.1572>
- Shah, K. and Maghsoudlou, P., 2016. Enzyme-linked immunosorbent assay (ELISA): the basics. *British Journal of Hospital Medicine* 77: 98101. <https://doi.org/10.12968/hmed.2016.77.7.C98>
- Shen, G., Kang, X., Su, J., Qiu, J., Liu, X., Xu, J., Shi, J. and Mohamed, S.R., 2022. Rapid detection of fumonisin B₁ and B₂ in ground corn samples using smartphone-controlled portable near-infrared spectrometry and chemometrics. *Food Chemistry* 384: 132487. <https://doi.org/10.1016/j.foodchem.2022.132487>
- Sibanda, L., McCallum, K., Plotan, M., Webb, S., Snodgras, B., Muenks, Q., Porter, J. and Fitzgerald, P., 2022. Interlaboratory collaboration to determine the performance of the Randox food diagnostics biochip array technology for the simultaneous quantitative detection of seven mycotoxins in feed. *World Mycotoxin Journal* 15: 241-250. <https://doi.org/10.3920/WMJ2021.2696>
- Streit, E., Naehrer, K., Rodrigues, I. and Schatzmayer, G., 2013. Mycotoxin occurrence in feed and feed raw materials worldwide: long-term analysis with special focus on Europe and Asia. *Journal of the Science of Food and Agriculture* 93: 2892-2899. <https://doi.org/10.1002/jsfa.6225>
- Te Kulve, H. and Rip, A., 2011. Constructing productive engagement: pre-engagement tools for emerging technologies. *Science and Engineering Ethics* 17: 699-714. <https://doi.org/10.1007/s11948-011-9304-0>
- Tittlemier, S., Cramer, B., Dall'Asta, C., DeRosa, M., Lattanzio, V., Malone, R., Maragos, C., Stranska, M. and Sumarah, M., 2022. Developments in mycotoxin analysis: an update for

- 2020-2021. *World Mycotoxin Journal* 15: 3-25. <https://doi.org/10.3920/WMJ2021.2752>
- Umapathi, R., Ghoreishian, S.M., Sonwal, S., Rani, G.M. and Huh, Y.S., 2022. Portable electrochemical sensing methodologies for on-site detection of pesticide residues in fruits and vegetables. *Coordination Chemistry Reviews* 453: 214305. <https://doi.org/10.1016/j.ccr.2021.214305>
- Wagacha, J.M. and Muthomi, J.W., 2008. Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology* 124: 1-12. <https://doi.org/10.1016/j.ijfoodmicro.2008.01.008>
- Zhou, S., Xu, L., Kuang, H., Xiao, J. and Xu, C., 2020. Immunoassays for rapid mycotoxin detection: state of the art. *Analyst* 145: 7088-7102. <https://doi.org/10.1039/D0AN01408G>