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Recent Strategies for Microbial and Fungal Decontamination of Cereals with a Potential for Application in Food Production

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Abstract

Microbial contamination of crops and plants during the growth, harvesting, and storage could cause significant economic losses because of their impact on food quality and safety, and animal health and productivity. There are few possibilities to avoid contamination of food/feed such as prevention of contamination, inhibition of fungal and/or bacterial growth during harvest and storage, precise sampling and monitoring of toxins levels, and microbial decontamination during food/feed processing by using novel tools. The integrating detection and mitigation of contaminationand evaluation of processing technologies for decontamination and detoxificationor high-risk commodities should be a priority.

Keywords: *Cereals; mycotoxins; acoustic sensing; ozonation; lactic acid fermentation; antimicrobial products.*

Introduction

My co toxins cause significant economic losses because of their impact on agricultural crops, food quality and safety, and animal health and productivity. The economically most important mycotoxins in foods and feeds are aflatoxins and ochratoxins produced mainly by Aspergillus speciesand Fusarium toxins (trichothecenes, zearalenone, and fumonisins). Principally, there are few possibilities to avoid contamination of food and feed caused by mycotoxins: prevention of contamination, inhibition of fungal growth during harvest and storage, precise sampling and monitoring of mycotoxin levels, and decontamination of mycotoxins during food and feed processing (Varga and Tóth, 2005; Kabak *et al*., 2006; Milani and Maleki, 2014). Although the different strategies used at present have been to some extent successful, most of them have major disadvantages, starting with mycotoxin resistance to thermal treatment, limited efficacy to losses of important nutrients and generally with high costs.

A plenty of various types of plants due to their nutritional, antioxidant and antimicrobial properties can be exploited for food applications. Plant material is attackable to microbial contamination during the growth, harvesting, and storage, which significantly affected the quality and shelf life of the products. Thus, decontamination of savory plants eliminating pathogens and reducing the number of spoilage microorganisms is necessary. The different tools including steaming, fumigation, and irradiation have been used for microbial decontamination with various degrees of success (Fowles *et al*., 2001; Lee *et al*., 2004; Sharma *et al*., 2003).

The integrating evaluation of processing technologies for their impact on microbial contamination and development of detoxification technologies for high-risk commodities should be a priority. While physical techniques currently offer the most efficient post-harvest reduction of microbial spoilage in food, biotechnological means possesses the largest potential for future developments.

Rapid Screening Of Mycotoxins In Cereal Grain

Acoustic Screening of Mycotoxins In Cereal Grain

The European Union (EU) has been working for almost two decades on the harmonization of my cotoxin standards for cereals and foods, based on international toxicological analysis (Van

Egmont *et al*., 2007) and established regulations including limits for the commodities and methods of analysis and sampling (FAO, 2004). Because of the high cost of rather slow and expensive wet chemistry methods and the fact that industry has been reluctant to carry out representative sampling for food safety purposes, the EU was felt obliged to enforce rather rigid sampling measures (EC 401/2006). Until now representative sampling of grain batch in the way the EU has enforced brings scarcely protection to the consumer and producer.

The rapid methods of mycotoxin analysis in cereals for food and feed are required to assess the toxicological effect on the humans and animals health. The diffuse reflectance UV spectroscopy or near infrared (NIR) spectroscopy appear to provide a new approach for deoxynivalenol (DON) evaluation in contaminated grain (Ruan *et al*., 2001; Stroka *et al*., 2004). According to these authors, the combination of a neural network and NIR spectra could be convenient to determine DON levels in food matrices. Though, a non-invasive technique with a satisfactory correlation of relevant parameters, which only needs one step, can give closer to the true values of the batch being investigated (Ruan *et al*., 2001; Siuda *et al*., 2008). In recent years, the potential of using IR spectroscopy for the detection of mycotoxins, including DON, ochratoxin, fumonisins and aflatoxins, and fungal contamination in cereals and cereal products has been also demonstrated (Peiris *et al*., 2009; De Girolamo *et al*., 2009). However, the technique is not a particularly proper for routine batch analysis because of the limited applications. The main disadvantages of IR instruments are the slow scan speed and low sensitivity (Pasquini, 2003).

The limitations of NIR and UV-vis spectroscopy and optical methods inspired to apply acoustic waves for quantitative DON determination directly on mixtures of unaffected (wholesome) and affected (contaminated/scabby) grains and aflatoxin inoculated corn kernels (Juodeikiene *et al*., 2009a). The speed of an acoustic method and the non-invasive character of the test that's quite possible to use in-line high-throughput analysis of grain as was tried with NIR. Recent developments (Pascale, 2009) cannot beat the speed of acoustic‘s because it is all two-dimensional techniques applied on a mono-layer of grains and/or time-consuming wet chemistry techniques that need a time-consuming extraction and clean-up phase, while acoustic‘s is three-dimensional (multi-layer).

Tutelyan (2004), who determined a direct correlation coefficient between 0.80 and 0.96 in DON concentration and the percentage of scabby grains with chemistry methods, triggered the increase in the quantitative development of the acoustic method for this application. These relations play an important role in the quantitative evaluation of DON using the acoustic sensing. As the acoustic signal propagates through or is reflected by the matrix, any texture changes affect the sound velocity and fixed signal amplitude (Kulmyrzaev *et al*., 2000; Stroka *et al*., 2004). Strong correlations obtained between the total concentration of Fusarium toxins (DON, ZEA, and T-2/HT-2) determined by ELISA and calculated based on the linearity of acoustic measurement shows the potential of this technique to give an overview of the infection of grains by Fusarium spp. (Juodeikiene *et al*., 2008, 2011). Furthermore, high correlations ($r = 0.84$) found between the content of corn seeds contaminated by Aspergillus flavus in the corn mixtures and ZEA concentration and the acoustic signal parameters confirm the acoustic method giving sufficiently good results in the quantitative determination of aflatoxin in corn, as well (Juodeikiene *et al*., 2009a). A single laboratory validation revealed the precise performance characteristics of the acoustic measurements (Juodeikiene *et al*., 2014) in terms of reproducibility (Thomson *et al*., 2002). Thus, the new approach could be advantageous for the screening of my cotoxin contamination within regulations (EC1881/2006) with DON detection limit of ≥200 ppb, and could meet a part of the problems commonly associated with sampling and analysis of grain (Juodeikiene *et al*., 2014).

Inhibition of Fungal And Microbial Growth In Cereals And Plants by Lactic Acid Bacteria

Recently, the use of biological decontamination/ biodegradation with microorganisms or enzymes leads to decontamination without significant losses in nutritive value and palatability of food and feed (Shetty and Jespersen, 2006; Dalie *et al*., 2009; Juodeikiene *et al*., 2009a). Biopreservation refers to the extended shelf life and enhances the safety of foods obtained by using natural or added microflora and antimicrobial metabolites.

A development of lactic acid bacteria (LAB) strains with antimicrobial effect against various bacteria and even food pathogens, e.g., Listeria, Salmonella, and Escherichia is of great importance (Cizeikiene *et al*., 2013). The antimicrobial properties of microorganisms give the basis for expanding its scope by using antimicrobial products not only in the food industry but also in other fields, especially for the reduction of biological pollution of grain seeds in organic farming (Supronieneet *al*., 2015) and in malt production, as well.

Biological treatments for the decontamination of grain seeds or malting grains should be not only effective but also cheap. One of the industrial waste has attracted much attention is a by-product of dairy industry – cheese whey permeate (Juodeikiene *et al*., 2016a). The study of the application of LAB antimicrobial bio-products suitable for microbial decontamination without the negative effect on grains and plantsis of outstanding importance. The treatment of malting wheat grain by LAB fermented cheese whey could lower not only the DON and ZEA contents by 38-47% but also to reduce the T-2/TH-2 toxin levels up to 62% (Juodeikiene *et al*., 2016b). The effect of bio-treatment on grain germination energy depends on the grain contamination level and LAB strain used for the bio-product fermentation (Suproniene *et al*., 2015). The metabolites produced by Lactobacillus and Pediococcusspp. strains show the fungicidal activity against Fusarium culmorum, F. poae, and F. solani, also the fungistatic activity against F. avenaceum, and F. solani (Suproniene *et al*., 2015; Juodeikiene *et al*., 2016b).

Herbs and spices have been recognized as natural food additives and applied not only for food aromatization but also preservation (Park *et al*., 2013; Libran *et al*., 2013). One of the modernapproaches to improve the safety and functional value of food products could be the combined application of anti microbial properties of savoury plants and bacteriocins producing LAB in food production.The addition of Silybummarianum seeds fermented with pediococci could significantly decrease the bacterial spoilage of bread (Juodeikiene *et al*., 2013). The fermentation of Satureamontana and Rhaponticumcarthamoides plants with LAB strains producing bacteriocins can remarkably reduces the amount of, yeast, fungi, enter bacteria and spores of mesophilic bacteria (Mozuriene *et al*., 2016).

Microorganisms showing a broad range spectrum of activity against fungal and microbial growth, therefore, are potential in various food production processes to prevent food spoilage and an increase of food safety.

Recent Methods For Decontamination Of Cereal Grain And Plants

Although preventive methods to control the mycotoxins in unprocessed grain are applied, there is an increasing awareness to control contamination with mycotoxins during storage, so safe and effective technologies are increasingly needed. The use of gaseous ozone as a powerful antimicrobial agent for cereal grain preservation could be of high practical importance in storage and processing (Khadre *et al*., 2001). Many recent investigations have shown the advances in using ozone gas for degradation of mycotoxins, such as aflatoxins (Inan *et al*., 2007) and Fusarium mycotoxins(Li *et al*., 2015) during storage. High oxidizing capacity allows the application of ozone gas in the destruction of bacteria, viruses, fungi, and fungal spores in cereals and food products (Raila *et al*., 2006). The effectiveness of ozone depends on several factors, including grain mass, temperature, moisture and the grain surface characteristics (Savi *et al*., 2014). Ozone could reduce aflatoxin levels up to 77% after treatment at 75 °C for 10 min (Proctor *et al*., 2004), or up to 93% after exposure to 66 mg/L O3 for 60 min (Inan *et al*., 2007). Ozone treatment (20 mg/L; exposure 80 min) indicates a positive influence on the degradation rate of trichothecenes in malting wheat grain, depending on the mycotoxin type and their initial contamination level (Reinholds *et al*., 2016). Ozone gas (exposure of 60 μmol/mol for 120 min) is effective in the inactivation of *F. graminearum* not altering physical and biochemical changes on the wheat grain (Savi *et al*., 2014). Since the scope for mycotoxin decontamination is still very limited, therefore, the elimination of mycotoxins from foods/feedstuffs is a big problem worldwide. Ozone as a natural agent may offer unique advantages for grain processing along with addressing growing concerns over the use of harmful pesticides.

Recently, considerable interest has arisen in the application of pulsed ultrasound as one of the alternative technological means which has a potential for the bacterial decontamination of fermentation media. The S. marianum plant seeds exposition to ultrasonic vibrations at the frequency of 20 kHz for280 sallow to reduce up to 50% of the total amount of microorganisms in the plant material and up to 68.8% in the fermented by LAB plant products used as supplements for functional bread (Juodeikiene *et al*., 2013). However, there is a lack of published data on the antimicrobial effect of ultrasound treatment on plant products in combination with lacto-fermentation. When assessing the potential safety problems, the search for new biotechnological tools to eliminate microbial contamination from cereal or plant products is especially relevant.

CONCLUSIONS

Fusarium mycotoxin DON contamination seems to be an important issue in human and especially in animal nutrition worldwide. Several strategies can be undertaken to reduce the toxic effect of DON. As a preventive mean, it is possible to use an acoustic sensing for cereal grain monitoring which could help to ensure that contents of contaminated grains will be removed from food/feed processing chain. The method originally developed for testing the structure of porous and aerated food products has also given reliable results for the quantitative determination of certain mycotoxin in grain and is sufficiently precise to meet the performance characteristics of wet chemistry methods as defined in the EU. The method is by far the fastest and cheapest method and is an excellent tool for monitoring and offers a high-throughput possibility for measuring and screening mycotoxins in cereals. Because of on-line capabilities and spatial action, it is able as to handle higher quantity, which draw representative sampling of grain.

Currently, mycotoxins are partially controlled through breeding plants for resistance to fungal diseases, fungicide treatment, and various agronomic practices. Biotechnology may help to provide additional protection against mycotoxins, furthermore, the biological methods are the most promising for the practical application of detoxification processes taking place directly in the feed production, although this approach is still in its infancy. A broad range of antifungal activity of LAB metabolites produced in certain fermentation medium show the potential of such antimicrobial products to be used as an effective tool for fungal decontamination in food production chain. Moreover, a strategy that is suitable for economically feasible testing and treatment of cereals providing safe food and feed products should be developed.

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