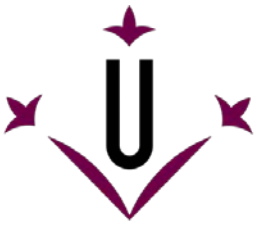

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Highlights

- Postharvest mycotoxins contamination represents a worldwide problem.
- Predictive models on mycotoxin production in foods are scarce.
- Luedeking-Piret, Baranyi, polynomial and logistic models are the main mathematical approaches.
- A number of challenges need to be solved before they are applied in food safety management.

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Modeling postharvest mycotoxins in foods: recent research

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Abstract

Available information on the prediction of postharvest production of mycotoxins in recent years is reviewed. Predictive mycology has been focused mainly on fungal growth whereas studies on prediction of mycotoxins in foods are scarce. Modeling mycotoxin production is challenging due to the high variability in mycotoxigenic potential among species and isolates. Besides mycotoxin biosynthesis pathways and factors influencing them are still poorly understood. Baranyi and Luedeking-Piret models have been recently used as primary models for mycotoxin prediction, while for secondary modeling, polynomial approaches have been used. Furthermore, probability models can be a different alternative. In any case, media for data generation, intraspecies variability, and microbial interactions should not be disregarded before model application in food safety management systems.

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INTRODUCTION

Food industry aims to obtain good quality and safe products and to maintain this throughout their shelf-life. Nevertheless, mycotoxins, as natural contaminants are not easy to control for both producers and exporters. Mycotoxins are secondary metabolites, toxic to human and animal health, produced by a wide range of fungi. Mycotoxins contamination represents a worldwide problem in terms of human/animal health and furthermore can pose a heavy economic burden to the industry. Mycotoxins can contaminate a product all over the food chain, in the field as well as during storage, or at later points (figure 1). Herein we will focus on the postharvest stage, where many factors are involved in the production of each particular mycotoxin; (a) intrinsic nutritional factors, (b) extrinsic factors, (c), processing factors and (d) implicit microbial factors [1].

As a result of inadequate handling/logistic structures, fungal growth and subsequent mycotoxin production are allowed. While the complete elimination of mycotoxin in contaminated foodstuffs is not achievable at this time, the aim is to focus on minimizing the occurrence of these toxins throughout the food chain. The implementation of good manufacturing practices (GMP) during handling, storage, processing and distribution represents an important line of defense in controlling the postharvest contamination of commodities by mycotoxins. To date, several postharvest strategies to prevent/reduce growth and mycotoxin production have been proposed. It is clear for the industry that drying of cereals and nuts, and temperature and moisture control during storage are factors of great importance, and other techniques including the application of compounds with antifungal effects such as synthetic antioxidants, essential oils [2,3], salts [4], natural phenolic compounds [5], or the use of modified atmospheres [6] have been used. In the past few years, there have been an increasing number of studies dealing with the use of bacteria, yeasts and moulds to control mycotoxigenic moulds in foods [7,8]. Beyond this, predictive mycology, providing tools for the prediction of fungal growth and mycotoxin production [9,10*], seems to be a promising approach and could play a role in improving the quality and safety of food. This tool may help for adequate decision making purposes, risk assessment and in the implementation of mitigation strategies.

POSTHARVEST MYCOTOXINS

Many fungi can invade and cause damage to grains, seeds, raw materials and different foods and feeds during transport and storage steps, either before or after drying. Particular postharvest practices which may be conducive to toxin accumulation and need further control and prevention strategies are slow

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drying of fruits in certain areas [11] or postharvest ensilage of dairy cow feed materials [12]. *Aspergillus* and *Penicillium* are the major mycotoxigenic postharvest fungi. The minimal necessary water activity (a_w) for most *Aspergillus* and *Penicillium* species is 0.75–0.85, but they can grow optimally at a_w 0.93–0.98. These fungi can grow at temperatures between 25-40 °C [13,14]. Typical postharvest mycotoxins are ochratoxin A (OTA), aflatoxins (AFs) (also typical in preharvest) and, in to lesser extent, deoxynivalenol (DON) [15]. A special reference must be made to patulin, which is an exclusively postharvest mycotoxin which affects fruits, mainly apples. This review deals, however, with OTA and AFs in which more attention has been focused lately.

RECENT RESEARCH ON PREDICTIVE MODELING OF POSTHARVEST MYCOTOXINS PRODUCTION

Postharvest modeling tries to simulate the conditions to which food would be exposed in order to forecast the microbial behavior and then to optimize postharvest management. Detailed predictive studies on mycotoxin production under various storage conditions are limited, but there is a wealth of information aiming to predict the growth of mycotoxigenic fungi and the influence of environmental factors on it. During the past decade several publications have dealt with the production of different mycotoxins over time, nevertheless, these studies rarely took into account the possibility to model such production. From the food safety point of view the target to be modeled are the mycotoxins, however modeling mycotoxin concentration could be an unpractical approach due to the high variability in mycotoxin potential among species and even more, among strains [16**]. The mycotoxins modeled and the models used in the existing studies presented in the following sections are listed in table 1.

MODELING AFLATOXINS

Although AFs are a common problem at harvest, the situation may worsen during postharvest when foodstuffs are stored under conditions that promote the growth of the specific microorganisms that produce them, *Aspergillus flavus* and *A. parasiticus*, which primarily contaminate food crops such as maize, peanuts, and tree nuts in tropical and subtropical climates.

Regarding AFs, several studies are available which model the effect of a_w and temperature on synthetic media [17*] and on real food matrices like maize grain [18] or pistachio nuts [19,20**]. Recently, Baranyi and Luedeking-Piret models have been the two primary models used to predict aflatoxin (AF) production over time. Garcia et al. [18] modeled the kinetics of aflatoxin B_1 (AFB1) production by *A.*

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flavus using the general mixed-growth associated Luedeking-Piret model for product formation under the assumption that both no-growth-associated and growth-associated toxin production existed. They considered three possibilities to estimate AFs formation, namely through colony radius, colony surface or biomass dry weight, demonstrating that AFs were produced during active growth of the fungus and when the growth had stopped, therefore AF biosynthesis did not present a clear delay in relation to growth. In such approach, parameters α and β are estimated, where α is the growth-associated coefficient for toxin production (g toxin /g biomass) and β is the non-growth-associated coefficient for toxin production (g toxin/g biomass per unit of time). This kind of modeling allows for some understanding of the global physiology of fungi, and it would be of interest to know the variation of these parameters as a function of environmental conditions. Later, Lee et al. [17*] estimated the maximum AFs production rate (ng/day), and the lag phase duration for AFs (day) by fitting the primary model of Baranyi to the production of AFs with respect to time. In this approach, toxin production is modeled independently of the coexisting fungal growth, thus the estimated rate accounts for both the increase in toxin linked to growth plus the increase due to already existing biomass, but no information is given by the model on each contribution.

Secondary modeling of AFs has been carried out by polynomial approaches. Lee et al. [17*] employed Gaussian and polynomial models to fit the maximum specific AFs production rate and the lag phase duration for AFs production, respectively, to describe the effects of a_w and temperature on these kinetic parameters. Interestingly, Medina et al. [21**] linked AFs production with gene expression by developing a modified Luedeking-Piret model including gene expression of AFB1 production, temperature, a_w and growth rate. This new approach gives a helpful understanding on the relationship between environmental stressing factors and the genes involved in the biosynthetic pathways of mycotoxins production, and may allow for refining of the existing models, through tuning of the potential for toxin production depending on gene activation.

Probability models have rarely been used to model mycotoxin production. Due to the high variability of toxin production (concentration) among strains, the predicted concentration from a model developed with one/various strains may not be applicable to other strains existing in nature, thus an alternative might be probability models, if they are proved not to be strain-dependent. Marín et al. [19] obtained probabilistic models (toxin/no-toxin) to predict AFB1 production by *A. flavus* including % moisture content, temperature and time. They converted the AFB1 experimental data into probabilities of AFB1 contamination by assigning 1 to samples with AFB1 presence and 0 to those without AFB1 (threshold of

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153 presence was established by the limit of detection of the equipment). Afterwards linear logistic
154 regression was applied to obtain the probabilistic model for AFB1 production. In this case, instead of
155 predicting the toxin concentration produced over time, the probability of toxin production is obtained,
156 thus, for example to avoid the risk of toxin accumulation in the storage a probability under 0.5 or 0.10
157 should be achieved through temperature and humidity control of the storage.
158
159 For application of these models to food and feed safety management in postharvest operations, there is
160 a need to go a step further and work on predictions under variable temperature/water activity
161 scenarios. In a preliminary study, Aldars-García et al. [20**] attempted to predict AFB1 formation under
162 a changing temperature environment, using probabilistic models too, for the prediction of AFB1
163 presence in pistachio nuts. They developed predictive models that could predict the presence of AFB1 in
164 pistachio nuts under a changing profile of temperatures with 67-81 % of concordance between
165 observed and predicted data, depending on the profiles.
166
167 MODELING OCHRATOXIN A
168 OTA is a mycotoxin of major concern which can be produced by several species of *Aspergillus* and
169 *Penicillium* species [22], and it is a common natural postharvest contaminant in cereals, nuts, dried
170 fruits, spices, etc. Few studies modeled the production of this toxin, as most of them just quantified the
171 toxin, either at various time points or at a single incubation time, and related it to the modeled growth
172 of the mould [23,24].
173 Kapetanakou et al. [25], including the viscosity of the substrate in their experimental design, modeled
174 the effect of temperature, a_w and (gel) structure on OTA production on malt extract broth and food
175 matrices with different viscosities. The Baranyi model was applied to estimate the OTA production
176 kinetic parameters, namely OTA production rate (ppm/day) and total toxin accumulation (ppm),
177 showing good fitting to the experimental data. The Luedeking-Piret model was applied by Lappa et al.
178 [16**] to assess the differences in OTA production among ten different strains of *A. carbonarius* isolated
179 from Greek vineyards. As Garcia et al. [18] did, they firstly determined fungal growth parameters and
180 correlated them with OTA, and finally took into consideration those growth parameters with the highest
181 correlation with OTA, i.e. colony diameter, colony area and biomass dry weight, excluding colony
182 density. Further they used them to model the amount of OTA produced in relation to incubation time,
183 concluding that OTA was a mixed-growth associated metabolite of *A. carbonarius*; this would support

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its early accumulation in fungal cultures. Besides, OTA production revealed a wide dispersion among isolates, pointing out the importance of taking into account the intraspecies variability in the predictive models.

Ioannidis et al. [26*] studied the effect of sodium metabisulphite (NaMBS) as a control technique in grapes during postharvest. OTA production over time was modeled with linear primary model to estimate the OTA production rate. To fit the model, they plotted the OTA concentrations against sampling times (3, 7, 10, 14, 17 days). However, in most of the cases a decrease in OTA amount was detected in the last two OTA sampling points, thus these points were excluded from the regression, to take into account only the linear part. Existing studies on most mycotoxins have shown that toxin concentration in open solid systems usually increases with time till a plateau is reached and sometimes a decrease is observed; however there are no concluding works on how and why degradation takes place. It is a pity that some of the latest studies on primarily modeling of toxins did not include the plots of their raw data over time, as there is a lack of availability of such data in order to decide on which primary model should be used.

Finally, as for AFs, polynomial models are the main mathematical tools used for secondary modeling of OTA formation under different environmental conditions. Kapetanakou et al. [25] modeled the square root of the OTA production rate using a polynomial model and a cardinal model. Nonetheless, the latter model showed poor adjustment possibly due to the narrow range of temperatures and a_w assessed in the experiment. Using a quadratic polynomial model, Ioannidis et al. [26*] described the effect of temperature, a_w, NaMBS concentration on the OTA production rate by *A. carbonarius* on grape juice based medium. The statistical indices used to assess the goodness of fit of the models displayed the difficulty of predicting the toxin formation in comparison with growth parameters.

CONCLUSIONS

Postharvest mycotoxins pose a threat for the safety of food products during transport, storage and distribution. Despite the high cost of data generation, and the challenging variability of mycotoxin data, a significant effort for developing predictive models for estimating mycotoxin contamination has been made during the past years and it is still in progress. There are a number of points which still need to be addressed:

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(i) Most models include a_w and temperature as the most critical factors which determine mycotoxin production. It is known that pH plays a minor role in most food and feed materials, however, it would be of interest to take into account in the models the impact of microbial interactions, which may be the main source of biased predictions.

(ii) Mycotoxin production in agar systems is quite different from that in real food and feed matrices, there is an urgent need for validation of the developed models in real substrates; or even to generate the data directly in foods and feeds.

(iii) Intraspecific variability in mycotoxin production is still a challenge, if even probability models result to be strain-dependent, the last resource would be to use growth models to predict growth boundaries and apply them to prevent toxin production, in a worst scenario approach.

Overall, the prediction of the accumulation of mycotoxins in foods and feeds is a challenging task due to the variety of factors influencing their production such as temperature, a_w, inhibitors, fungal strains, accompanying microbiota, etc., and the need to understand the mycotoxin biosynthesis more deeply.

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* of special interest

** of outstanding interest

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Table 1. Recent predictive mycotoxin models used in food mycology.

Reference	Type of model			Mycotoxin
	Primary	Secondary	Probabilistic	
García et al. [18]	Luedeking-Piret	-	-	AF
Lee et al. [17*]	Baranyi	Gaussian and polynomial	-	
Medina et al. [21**]	Luedeking-Piret	-	-	
Marín et al. [19]	-	-	Logistic	
Aldars- García et al. [20**]	-	-	Logistic	
Kapetanakou et al. [25]	Baranyi	Polynomial	-	OTA
Ioannidis et al. [26*]	Linear	Polynomial	-	
Lappa et al. [16**]	Luedeking-Piret	-	-	

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FIGURE CAPTIONS

Figure 1. Brief description of the food chain and the main factors influencing fungal growth and mycotoxin production (Modified from Magan et al., [27]).

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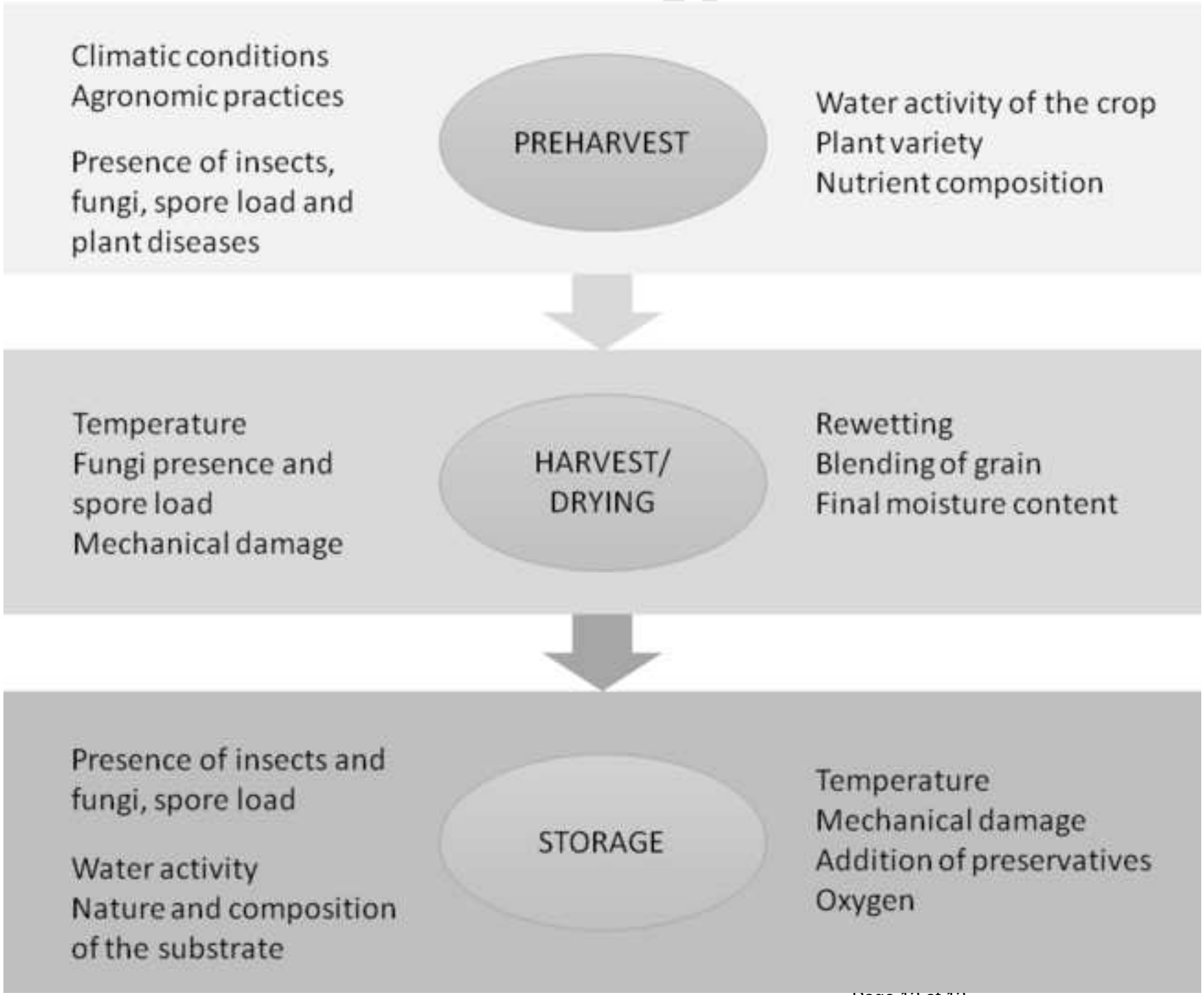
Figure


Climatic conditions
Agronomic practices
Presence of insects, fungi, spore load and plant diseases
PREHARVEST
Water activity of the crop
Plant variety
Nutrient composition
Temperature
Fungi presence and spore load
Mechanical damage
HARVEST/ DRYING
Rewetting
Blending of grain
Final moisture content
Presence of insects and fungi, spore load
Water activity
Nature and composition of the substrate
STORAGE
Temperature
Mechanical damage
Addition of preservatives
Oxygen