



Review

Predicting mycotoxins in foods: A review

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ABSTRACT

The need to ensure the microbiological quality and safety of food products has stimulated interest in the use of mathematical models for quantifying and predicting microbial behaviour. For 20 years, predictive microbiology has been developed for predicting the occurrence of food-borne pathogens, although these tools are dedicated to bacteria. Recently, the situation has changed and a growing number of studies are available in the literature dealing with the predictive modelling approach of fungi. To our knowledge the present one is the first review focussed on predictive mycology and food safety, including mycotoxins; existing kinetic and probability models applied to mycotoxigenic fungi germination and growth, and mycotoxin production are reviewed.

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1. Introduction

Yeast and moulds are found in a wide range of environments due to their capacity to utilize a variety of substrates and to their relative tolerance to low pH, low water activity, and low temperature (Huis in't Veld, 1996). Generally, foods have essential nutrients for fungal growth, thus fungi can appear and spoil different foods and feeds. Fungal spoilage of food causes economics losses worldwide (Dantigny et al., 2005). In addition, some moulds synthesize toxins called mycotoxins, which can be a hazard for human health. Pitt (1996) defined mycotoxins as fungal metabolites that when ingested, inhaled or absorbed through the skin cause illness or human and animal death. Mycotoxins are natural contaminants in raw materials, foods and feeds. Some mycotoxins can cause autoimmune illnesses, have allergenic properties, and some of them are teratogenic, carcinogenic, mutagenic, etc. (CAST, 2003). From the food safety point of view, only mycotoxins, as chemical hazards, are important, while moulds and yeast themselves may cause spoilage but have no safety implications.

The need to ensure the microbiological quality and safety of food products has stimulated interest in the use of mathematical models for quantifying and predicting microbial behaviour (Lahlali et al., 2005). For 20 years, predictive microbiology has been developed for predicting the occurrence of food-borne pathogens, although these tools are dedicated to bacteria (Dantigny et al., 2005). Recently, the situation has changed and a growing number

of studies are available in the literature dealing with the predictive modelling approach of fungi (Parra and Magan, 2004; Patriarca et al., 2001). Since 1995, there have been an increasing number of publications dealing with modelling of mould growth in relation to food (Scopus database, Fig. 1). Some efforts have been devoted to prevent food spoilage, for example, in bakery products, but those models have no application for food safety issues. Fig. 1 excludes those publications regarding modelling of fungal production for food purposes.

Predictive modelling of filamentous fungal growth has not received the same attention as that of bacterial development. Gibson and Hocking (1997) cited this may be because of the inherent complexities associated with the quantification of fungal growth. While bacteria reproduce by fission, and normally grow homogeneously through a liquid medium or take place only at surfaces, fungal hyphae can penetrate the physical three-dimensional matrix of food (Dantigny et al., 2005). Bacteria form single cells and they can be easily enumerated, especially in liquid broth. At low cellular densities and in solid media, colony-forming units (CFU/ml or CFU/g) can be determined (Dantigny et al., 2005). Bacterial CFUs increase exponentially, and when transformed to log CFU bacterial growth can be modelled over time as the known sigmoidal growth curve, with or without upper (stationary phase) and lower (lag phase) asymptotes. However, moulds are not unicellular; they form mycelium whose weight, except at the early stage of growth, does not increase exponentially (Koch, 1975). Besides, it is difficult to determine the weight of the mycelium in a food matrix. In addition, it is impossible to split the mycelium into individual cells; therefore, the CFU method can be applied to the enumeration of spores

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Nomenclature			
a_w	water activity	λ_{germ}	lag phase prior germination (d, h)
$a_{w \text{ min}}$	a_w below which no growth occurs	pH_{min}	pH below which no growth occurs
$a_{w \text{ max}}$	a_w over which no growth occurs	pH_{max}	pH over which no growth occurs
$a_{w \text{ opt}}$	a_w at which μ_{max} is optimal	pH_{opt}	pH at which the μ_{max} is optimal
μ_{max}	maximum specific growth rate (it is defined as the slope of the growth curve at the point of inflexion) (mm/d, $\mu\text{m/h}$)	t	time (d, h)
μ_{opt}	maximum growth rate at optimal conditions (mm/d, $\mu\text{m/h}$)	T	temperature ($^{\circ}\text{C}$)
λ	lag period (defined as the intersection of the line defining the maximum specific growth rate with the x axis) (d, h)	T_{min}	temperature below which no growth occurs ($^{\circ}\text{C}$)
P	percentage of germinated spores (%)	T_{max}	temperature over which no growth occurs ($^{\circ}\text{C}$)
P_{max}	maximal percentage of germinated spores (%)	T_{opt}	T at which μ_{max} is optimal ($^{\circ}\text{C}$)
r	time where $P = P_{\text{max}}/2$ (d, h)	y_0	initial colony diameter (or radius), usually zero (μm , mm)
k	slope term at the point of inflexion for the rate of increasing germinated spores (d^{-1} , h^{-1})	y	colony diameter (or radius) (μm , mm)
		y_{max}	maximum colony diameter (or radius) attained, asymptotic value (μm , mm)
		N	number of spores
		N_0	initial number of spores
		$N(t)$	number of surviving spores after treatment
		k_i	inactivation rate (min^{-1})

only (Vindeløv and Arneborg, 2002). Measurement of hyphal extension rate, generally reported as radial growth rate in $\mu\text{m/h}$ or mm/d , is probably the most often used measurement, but it does not represent the true tridimensional fungal growth. Colony diameter is the most simple and direct form to measure mould growth (Gibson and Hocking, 1997) for estimating a growth rate parameter, as in general there is a linear increase of colony diameter over time.

The employment of models may be useful for decision-making purposes to prevent risks for human and animal health (Prandini et al., 2008). With these models the final levels of mould or mycotoxins contamination may be predicted. Predictive mycology thus constitutes a useful tool for the food industry. Membré and Lambert (2008) summarised some applications of predictive microbiology into three groups of activities related to food safety.

Product innovation: Assessing rate of microbial proliferation, growth limits, or inactivation rate associated with particular food formulations and/or process conditions in order to develop new products and processes, reformulate existing products, and determine storage conditions and shelf-life.

Operational support: Supporting food safety decisions that need to be made when implementing or running a food manufacturing

operation, such as designing in-factory heating regimes, setting critical control points (CCPs) in HACCP, assessing impact of process deviations on microbiological safety and quality of food products.

Incident support: Estimating the impact on consumer safety or product quality in case of problems with products on the market.

Besides predictive microbiology might be also utilized to transfer new risk management concepts into practical guidelines (Gorris, 2005; Membré et al., 2007).

To our knowledge, two reviews on predictive mycology have been previously published. The first one, 'Advances in the predictive modelling of fungal growth in food', dating from 1997, summarised the few existing works at that time (Gibson and Hocking, 1997). Later, Dantigny et al. (2005) in 'Basis of predictive mycology' published an updated review on the different primary and secondary models applied to germination and growth of spoilage fungi. To our knowledge the present one is the first review focussed on food safety, including mycotoxins; those works on modelling of fungal infection and mycotoxin accumulation as a function of field conditions during production of raw materials have not been considered here.

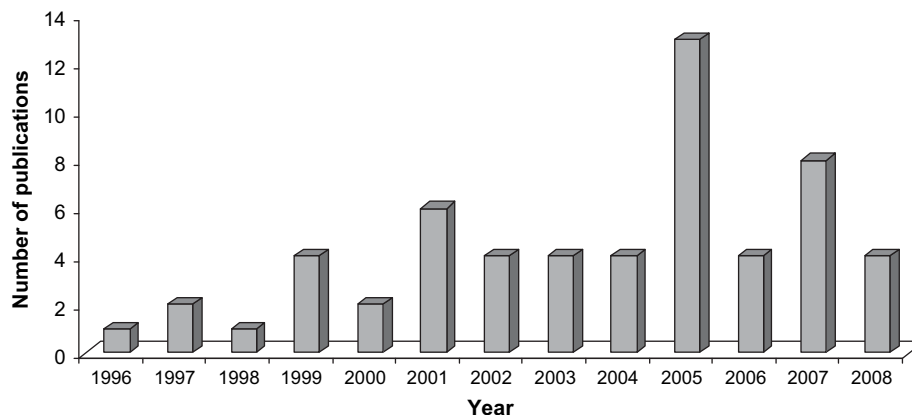


Fig. 1. Publications on modelling of food-borne mould growth in the last years (from Scopus database).

2. Kinetic predictive models applied to moulds germination, growth and inactivation

As mentioned before, mycotoxins are the target to be modelled from the food safety point of view. However, despite the absence of direct correlation between mould growth and mycotoxins production, the prevention of fungal growth in raw materials and foods leads invariably to the prevention of mycotoxins presence. Thus, modelling of germination and growth of toxigenic fungi may be a key alternative.

A model is “the description of a system, theory, or phenomenon that accounts for its known or inferred properties and may be used for further study of its characteristics”. The model is an often simplified description of relationships between observations of the system (responses) and the factors that are believed to cause the observed responses. Thus, a mathematical model can simply describe a collection of data or may represent a hypothesis or series of hypotheses about underlying relationships among the independent variables that lead to the observations or data (McMeekin et al., 2008).

In predictive microbiology, mathematical models are used to predict growth and toxins production by different microorganisms, and also to study the response of them to environmental factors. Microbial kinetic models can be classified as primary, secondary or tertiary (Whiting, 1995). Primary models describe how the number of microorganisms in a population change with time under specific conditions (Marks, 2007). Secondary models refer to primary model related to the influence of environmental factors, like water activity, pH, temperature, etc, in moulds germination and growth. Tertiary models combine primary and secondary models with a computer interface, providing a complete prediction tool (Marks, 2007). Due to the lack of specific models for moulds, there is a tendency to apply models that have been developed for bacteria.

2.1. Primary models

Models that describe the growth of a population of microorganisms over time form the basis for adopting strategies for food safety. As mentioned previously, there is a tendency to extend the use of models that were developed for bacteria to moulds, but an important specificity of fungi should be taken in account: spore germination (Prandini et al., 2008). Fungal growth involves germination and hyphal extension, eventually forming mycelium (Dantigny, et al., 2005). Primary microbial models can be classified as germination, growth and inactivation models (Soboleva et al., 2000).

2.1.1. Germination models

Fungal spores are disseminated in the environment and in contact with foods and feeds they can spoil them. Germination can be considered as the main step to be focused from the food quality point of view because a product is spoiled as soon as visible hyphae can be observed. A spore is considered to have germinated when the germinative tube measures twice the diameter of the initial spore. The germination occurs in a short time. There are few studies concerning germination kinetics; this shortage can be explained by the difficulties of acquiring sufficient, reproducible data (Dantigny, 2005).

Increase of percentage of germinated spores over time has been modelled through the Gompertz (Judet et al., 2008; Marín et al., 1996, 1998b; Pardo et al., 2004, 2005c,d, 2006a; Plaza et al., 2003) and the logistic model (Dantigny et al., 2002, 2005, 2006; Judet et al., 2008).

Logistic model

$$P = \frac{P_{\max}}{(1 + \exp(k(r - t)))} \quad (1)$$

Gompertz model

$$P = P_{\max} \exp \left[- \exp \left(\frac{k \exp(1)}{P_{\max}} (\lambda_{\text{germ}} - t) + 1 \right) \right] \quad (2)$$

A study was specifically designed to compare the performance of both models (Dantigny et al., 2007). Based on the root mean squared error (RMSE), the goodness of fit of both the models was similar. It was also impossible to determine for which of the moulds assayed and for which experimental conditions a particular model should be preferred. Therefore other criteria were also evaluated such as the accuracy of the parameter estimates. The germination time (time required for 50% of viable spores to germinate) was estimated with a greater accuracy by the logistic model than by the Gompertz equation, while lag phase was more accurately estimated by the Gompertz model. Care should be taken while using the Gompertz equation because in some cases erroneous estimations of the maximum percentage of germination (i.e., greater than 100%) were obtained.

2.1.2. Growth models

Once germination has taken place, hyphae elongate leading to mycelium spreading and macroscopic colonies formation. CFU counts, which resemble bacterial methods, may be applied to moulds, but they take into account spores number mostly. The most widely used method for modelling purposes is colony diameter measure because it is easier to obtain data, although it cannot be applied in food analysis. Ergosterol content has sometimes been used to quantify fungal growth, too (Marín et al., 2008a), as well as CFU/g in particulate or liquid substrates (Vindeløv and Arneborg, 2002). The advantages and drawbacks of methods used to quantify fungal growth of food-borne fungi were analysed by Marín et al. (2005). Mould growth in solid substrates (colony diameter) is generally assumed to follow a pattern consisting of a lag phase and lineal growth phase (or just a lineal phase) under optimal conditions with no limiting conditions (Fig. 2). Under suboptimal conditions a stationary phase may appear in which moulds stop growth (Gibson et al., 1994), for example under limiting conditions of water activity.

Some authors use sigmoidal models when their moulds, growing in Petri plates, describe a biphasic curve with lag and lineal phases, and they assimilate the stationary phase to the diameter of

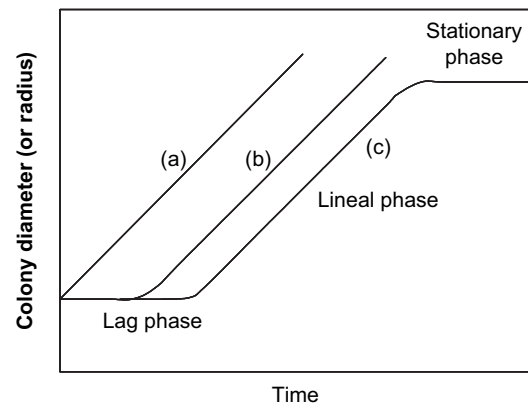


Fig. 2. Usual growth curves for moulds under optimal and suboptimal conditions. (a) Lineal model, (b) lineal model with lag, (c) sigmoidal model.

the Petri plate, but this latter value has no biological meaning, and this practice may affect the estimation of the other two curve parameters.

The most frequently used primary growth models are the lineal one, the Gompertz modified equation (Zwietering et al., 1990) and Baranyi and Roberts (1994) model (Table 1).

Lineal model

Lineal model has been the most widely model used to describe mycelial fungal growth in Petri plates. Growth rate can easily be calculated giving a useful parameter to compare among treatments in research trials, thus many studies other than the listed in Table 1 exist. Lag phase prior growth may be estimated through the interception between the regression line and the x axis. Lineal model is the easiest of the alternatives, but it must be restricted to conditions highly conducive to fungal growth. Under sub-optimal conditions, plotting of the results against time and manual (and subjective) selection of the 'straight' part of the line (avoiding lag phase and asymptotic one, if any) is required (Marín et al., 2008a).

Table 1
Examples of primary kinetic growth models in solid substrates used in food mycology.

Model	Fungal species	References
Baranyi	<i>Aspergillus</i> section <i>Flavi</i>	Gibson et al. (1994)
	<i>Penicillium roqueforti</i>	Valik et al. (1999),
	<i>Byssoschlamys fulva</i> , <i>Neosartorya fischeri</i> , <i>Talaromyces avellaneus</i>	Valik and Pieckova (2001),
	<i>P. brevicompactum</i>	Membré and Kubaczka (2000),
	<i>Monascus ruber</i>	Membré et al. (2001)
	<i>A. carbonarius</i>	Panagou et al. (2003)
	<i>Rhizopus oryzae</i>	Tassou et al. (2007)
	<i>A. flavus</i> , <i>A. parasiticus</i> , <i>Fusarium verticillioides</i> , <i>F. proliferatum</i>	Carrillo et al. (2007)
		Samapundo et al. (2005, 2007)
Gompertz	<i>Walleimia sebi</i>	Vindeløv and Arneborg (2002)
	<i>Eurotium chevalieri</i> , <i>A. fumigatus</i> , <i>P. brevicompactum</i>	Char et al. (2005)
	<i>P. expansum</i> , <i>A. carbonarius</i>	Marín et al. (2006a,b)
Linear	<i>F. verticillioides</i> , <i>F. proliferatum</i> , <i>A. ochraceus</i> , <i>A. flavus</i> , <i>A. niger</i> , <i>P. aurantiogriseum</i> , <i>P. hordei</i>	Marín et al. (1995, 1998b)
	<i>P. roqueforti</i> , <i>Trichoderma harzianum</i> , <i>Paecilomyces variotii</i> , <i>A. niger</i> , <i>Emericella nidulans</i>	Cuppers et al. (1997)
	<i>W. sebi</i>	Patriarca et al. (2001)
	<i>P. chrysogenum</i> , <i>A. flavus</i> , <i>Cladosporium cladosporioides</i> , <i>A. alternata</i> , <i>Mucor racemosus</i> , <i>R. oryzae</i> , <i>T. harzianum</i>	Sautour et al. (2001, 2002, 2003)
	<i>E. rubrum</i> , <i>E. repens</i> , <i>W. sebi</i> , <i>A. penicillioideis</i> , <i>P. roqueforti</i> , <i>Chrysosporium xerophilum</i> , <i>Xeromyces bisporus</i>	Gock et al. (2003)
	<i>F. verticillioides</i> , <i>F. proliferatum</i> , <i>F. graminearum</i>	Velluti et al. (2003, 2004a,b)
	<i>A. niger</i>	Parra and Magan (2004)
	<i>A. ochraceus</i> , <i>P. verrucosum</i>	Pardo et al. (2004, 2005a,b,c,d, 2006a)
	<i>A. section Nigri</i>	Bellí et al. (2004a,b, 2005)
	<i>P. verrucosum</i>	Cairns-Fuller et al. (2005)
	<i>F. culmorum</i> , <i>F. graminearum</i>	Hope et al. (2005)
	Range of 12 food spoilage fungi	Dantigny et al. (2005)
	<i>P. expansum</i> , <i>P. digitatum</i> , <i>P. italicum</i>	Lahlali et al. (2005, 2006)
	<i>P. expansum</i>	Baert et al. (2007)
	<i>A. carbonarius</i>	Medina et al. (2007)

Gompertz modified model

This is an exponential function that describes an asymmetrical sigmoidal curve. In the last years some researchers have used Gompertz modified equation by Zwietering et al. (1990), because the parameters have a biological interpretation:

$$y = y_{\max} * \exp \left\{ - \exp \left[\left(\frac{\mu_{\max} * \exp(1)}{y_{\max}} \right) (\lambda - t) + 1 \right] \right\} \quad (3)$$

y_{\max} , μ_{\max} and λ are the estimated growth parameters through the non-linear regression algorithm. y_0 is omitted in the equation, as assumed equal to zero.

Baranyi model

This is a widely used model for bacteria. The model assumes that, after a certain adjusting period, the growth rate is constant but an upper asymptote after the linear phase occurs.

$$y = y_0 + \mu_{\max} A - \ln \left\{ 1 + \frac{[\exp(\mu_{\max} A) - 1]}{\exp(y_{\max} - y_0)} \right\} \quad (4)$$

$$A = t + \left(\frac{1}{\mu_{\max}} \right) \ln [\exp(-\mu_{\max} t) + \exp(-\mu_{\max} \lambda) - \exp(-\mu_{\max} t - \mu_{\max} \lambda)] \quad (5)$$

y_0 , y_{\max} , μ_{\max} and λ are the estimated growth parameters through the non-linear regression algorithm. If this triphasic model wants to be converted to a biphasic (lag-linear) one, the last logarithmic term in Eq. (4) may be omitted, and then a growth function without upper asymptote can be obtained (Gibson et al., 1994). In most cases, mycelium is developing on an ever 'new' medium (i.e., the peripheral zone of the colony); there is no limitation to the growth due to the growth of the fungal colony due to substrate (Pirt, 1967), thus the Baranyi model would result in the linear model that has been used for many years by mycologists.

In 2004, López et al. (2004) underwent a statistical evaluation of mathematical models for microbial growth (measured as optical density units), including bacterial and fungal species. A range of models were considered, including Gompertz and Baranyi ones. The Baranyi model showed the best behaviour for the growth curves studied according to a variety of criteria. Their results indicated that the common use of the Gompertz model to describe microbial growth should be reconsidered critically, as the Baranyi, three-phase linear, Richards and Weibull models showed a significantly superior ability to fit experimental data than the extensively used Gompertz. The suitability of using either linear, Gompertz's or Baranyi's models for primary modelling of growth of a range of 14 common food spoilage fungal species was tested (Marín et al., 2008a). Regarding colony diameters, when no asymptotic trend was observed in the data, using either linear or Baranyi's function gave better estimations of maximum growth rate and lag phase. When a decrease in growth rate was observed with time, standard Baranyi's model was chosen. The use of Gompertz equation led, in general, to overestimated parameters.

As a conclusion, flexible Baranyi model can be applied to most situations, thus it is probably the best of the existing alternatives. There is a need also to standardise the use of either radius or diameter for modelling purposes; this would lead to an easier comparison among maximum growth rates published by different authors. The colony growth rate depends on the rate of elongation of the leading hyphae spanning the colony's peripheral zone (Trinci, 1971), thus the use of radius instead of diameter seems to be a more logical choice.

2.1.3. Inactivation models

Microbial populations can be subjected to a lethal treatment, like high temperatures or concentration of a lethal agent. Few studies have been published in modelling the inactivation of fungal spores, most of them used the linear inactivation approach, with the classical first order equation for the inactivation of spores:

$$\frac{dN}{dt} = -k_i N; \quad \ln N(t) - \ln N_0 = -k_i t \quad (6)$$

The D value or decimal reduction time is the time required to inactivate 90% of the spores at a given temperature, $D = (\ln 10/k)$. The z value ($^{\circ}\text{C}$), which is a secondary model, is the temperature increase required to have a 10-fold increase of the D value.

Published works on modelling of inactivation of fungal spores deal with thermotolerant ones, which cause common food spoilage such as *Byssoschlamys fulva*, *Byssoschlamys nivea*, *Talaromyces flavus* and *Neosartorya fischeri* (Bayne and Michener, 1979; Casella et al., 1990; Scott and Bernard, 1987). Only a few include mycotoxigenic fungi, mainly black aspergilli (Baggerman and Samson, 1988; Fujikawa and Itoh, 1996).

2.2. Secondary models

The growth of moulds in foods and feeds depends on the effects of multiple variables like pH, water activity (a_w), solute concentrations, temperature, etc. and time. Secondary models describe the influence of environmental factors on key parameters of the primary model; germination or growth parameters (e.g. maximum growth rate) estimated from primary models are then modelled as a function of intrinsic and extrinsic factors of foods. Two of the most important environmental parameters that determine the ability of moulds of growing in foods are water activity and temperature. In contrast to bacteria, water activity has a greater effect on mould

$$\mu_{\max} = \frac{\mu_{\text{opt}}(T - T_{\max})(T - T_{\min})^2}{(T_{\text{opt}} - T_{\min})\{(T_{\text{opt}} - T_{\min})(T - T_{\text{opt}}) - (T_{\text{opt}} - T_{\max})(T_{\text{opt}} + T_{\min} - 2T)\}} \quad (11)$$

$$\mu_{\max} = \frac{\mu_{\text{opt}}(a_w - a_{w(\max)})(a_w - a_{w(\min)})^2}{(a_{w(\text{opt}}) - a_{w(\min)})\{(a_{w(\text{opt}}) - a_{w(\min)})(a_w - a_{w(\text{opt}}) - (a_{w(\text{opt}}) - a_{w(\max)})(a_{w(\text{opt}}) + a_{w(\min)} - 2a_w)\}} \quad (12)$$

development than temperature (Holmquist et al., 1983). Few studies report on secondary modelling of germination data, and polynomial models were used in most of the cases (Halouat and Debevere, 1997).

Pitt (1993) developed a mechanistic model integrating literature data on the effect of temperature, a_w , pH, and colony size on *in vitro* mould growth of *A. flavus* and aflatoxin production. Some other empirical models have been later applied to fungi, most of them previously applied to bacteria. Most common secondary models applied to date to moulds are (Table 2):

2.2.1. Ratkowsky square-root model (Ratkowsky et al., 1983)

Originally developed for bacteria:

$$\sqrt{\mu_{\max}} = b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\} \quad (7)$$

b , c are estimated constants, as well as T_{\min} and T_{\max}

Originally this model included only temperature, but it has been applied to other factors such as a_w (Tassou et al., 2007):

$$\sqrt{\mu_{\max}} = b(a_w - a_{w \min})\{1 - \exp[c(a_w - a_{w \max})]\} \quad (8)$$

2.2.2. Linear Arrhenius–Davey equation (Davey, 1989)

Initially applied to model temperature effect on bacterial growth:

$$\ln \mu_{\max} = a_0 + a_1/T + a_2/T^2 \quad (9)$$

where T is absolute temperature (K). It may be extended to a_w and pH (Panagou et al., 2003):

$$\ln \mu_{\max} = a_0 + a_1 a_w + a_2 a_w^2 + a_3 \text{pH} + a_4 \text{pH}^2 + a_5/T + a_6/T^2 \quad (10)$$

$a_0, a_1, a_2, a_3, a_4, a_5, a_6$ are constants to be estimated

2.2.3. Rosso cardinal model

Rosso et al. (1993) proposed this temperature model that uses the three cardinal temperatures (T_{\min} , T_{\max} , T_{opt}) and the specific growth rate at the optimum temperature. This model was built empirically as a tool to describe data without the aim of a mechanistic explanation. A great advantage of this model is that all parameters have a physiological meaning which clearly facilitate initial parameter estimations and may also aid in future incorporation into the model of underlying cell biological mechanisms (Brul and Klis, 1999).

The model was some years later specifically tested on a data set of existing fungal growth data by Rosso and Robinson (2001), taking into account a_w as factor:

2.2.4. Gibson model (Gibson et al., 1994)

This model deserves a special mention because it was the first one developed specifically for moulds. Gibson et al. (1994) found that the logarithm of fungal growth rate showed a parabolic relationship with the square root of $(1 - a_w)$, resulting in a polynomial model that was applied to *A. flavus*:

$$\ln \mu_{\max} = a_0 + a_1 \sqrt{1 - a_w} + a_2(1 - a_w) \quad (13)$$

a_0, a_1, a_2 are constants to be estimated.

Table 2
Examples of secondary kinetic growth models in solid substrates used in food mycology.

Model	Fungal species	References	
Ratkowsky square-root	<i>Penicillium roqueforti</i> , <i>Trichoderma harzianum</i> , <i>Paecilomyces variotii</i> , <i>Aspergillus niger</i> , <i>Emericella nidulans</i>	Cuppers et al. (1997)	
	<i>A. niger</i>	Parra and Magan (2004)	
	<i>P. expansum</i>	Baert et al. (2007)	
Arrhenius–Davey	<i>Fusarium verticillioides</i> , <i>F. proliferatum</i>	Samapundo et al. (2005)	
	<i>P. expansum</i>	Baert et al. (2007)	
	<i>A. carbonarius</i>	Tassou et al. (2007)	
	<i>A. flavus</i> , <i>A. parasiticus</i>	Samapundo et al. (2007)	
Rosso cardinal	<i>P. roqueforti</i> , <i>T. harzianum</i> , <i>P. variotii</i> , <i>A. niger</i> , <i>E. nidulans</i>	Cuppers et al. (1997)	
	<i>P. chrysogenum</i> , <i>A. flavus</i> , <i>Cladosporium cladosporioides</i> , <i>Alternaria alternata</i> .	Sautour et al. (2001)	
	<i>A. flavus</i> , <i>A. nomius</i> , <i>A. oryzae</i> , <i>A. parasiticus</i> , <i>A. candidus</i> , <i>A. sydowii</i> , <i>E. amstelodami</i> , <i>E. chevalieri</i> , <i>Xeromyces bisporus</i>	Rosso and Robinson (2001)	
	<i>P. brevicompactum</i>	Membré et al. (2001)	
	<i>Monascus ruber</i>	Panagou et al. (2003)	
	<i>A. carbonarius</i>	Tassou et al. (2007)	
	Polynomial	<i>A. flavus</i>	Gibson et al. (1994)
		<i>P. variotii</i> , <i>A. niger</i> , <i>E. nidulans</i>	Cuppers et al. (1997)
		<i>P. roqueforti</i>	Valik et al. (1999)
		<i>P. chrysogenum</i>	Sautour et al. (2001)
		<i>Wallemia sebi</i>	Patriarca et al. (2001)
		<i>M. ruber</i>	Panagou et al. (2003)
		<i>A. niger</i>	Parra and Magan (2004)
<i>A. section Nigri</i>		Bellí et al. (2004a,b, 2005)	
<i>A. ochraceus</i> , <i>P. verrucosum</i>		Pardo et al. (2004, 2005a, b,c,d, 2006a,b)	
<i>F. verticillioides</i> , <i>F. proliferatum</i>		Samapundo et al. (2005)	
<i>P. expansum</i>	Lahlali et al. (2005)		
<i>E. chevalieri</i> , <i>A. fumigatus</i> , <i>P. brevicompactum</i>	Char et al. (2005)		
<i>A. carbonarius</i> , <i>P. expansum</i>	Marín et al. (2006a,b)		
<i>A. carbonarius</i>	Tassou et al. (2007)		
<i>P. expansum</i>	Baert et al. (2007)		
<i>M. ruber</i>	Panagou et al. (2007)		
<i>A. flavus</i> , <i>A. parasiticus</i>	Samapundo et al. (2007)		
Gamma Concept	<i>M. ruber</i>	Panagou et al. (2003)	
	<i>A. alternata</i> , <i>A. flavus</i> , <i>C. cladosporioides</i> , <i>Mucor racemosus</i> , <i>P. chrysogenum</i> , <i>Rhizopus oryzae</i> , <i>T. harzianum</i>	Sautour et al. (2003)	

It has also been extended to take into account the combined effect of temperature (Tassou et al., 2008):

$$\ln \mu_{\max} = a_0 + a_1 \sqrt{1 - a_w} + a_2(1 - a_w) + a_3T + a_4T^2 + a_5T(1 - a_w) \quad (14)$$

$a_0, a_1, a_2, a_3, a_4, a_5$ are constants to be estimated.

2.2.5. Other polynomial models

Polynomial models have been widely used in predictive microbiology for the quantitative assessment of the effects of various environmental factors on fungal growth (Table 2), generally a_w , temperature and pH. They are usually built through multiple linear regression analysis and allow any of the environmental parameters and their interactions to be taken into account. However, there are certain disadvantages of these models, such as:

- They are developed from linear and quadratic combinations of variables where linearity may not be justified.

- Collinearity problems among variables may exist.
- Sensitivity analysis of input variables is difficult due to the presence of crossinteractions.
- The parameters of the model have no biological meaning.

Polynomial models have been constructed to describe fungal germination and growth as a function of factors including water activity, temperature, pH, %O₂, %CO₂, preservatives concentration, etc. and their combined effects. The general expression of the polynomial model is:

$$Y = a_0 + \sum_{i=1}^3 a_i X_i + \sum_{i=1}^3 \sum_{j=1}^3 a_{ij} X_i X_j \quad (15)$$

a_i are the constants to be estimated

X_i are the independent or input variables (a_w , temperature...)

Y is the response variable (μ_{\max} , λ ...)

In practice, the choice of a transformation for the mould maximum growth rate depended upon the secondary model in the different published works. No transformation was used in general for modelling the effect of environmental factors using surface response methodology. Following the pioneer work of Gibson et al. (1994), many authors have used implicitly the logarithmic transformation for modelling the effect of $\sqrt{1 - a_w}$ by a simple quadratic function or in combination to other factors such as pH and temperature. Eventually, the logarithmic transformation was applied by using the extended Davey (1989) model, and the cardinal model (Rosso et al., 1993) or models derived from the square-root model (Ratkowsky et al., 1983). Studies that examined the homogeneity of the variance of mould growth data are scarce. In the objective of standardising methodologies within the emerging field of predictive mycology, Dantigny and Bensoussan (2008) aimed at determining a suitable transformation that can be used routinely a priori whatever the data. Their study suggests that the logarithmic transformation should be avoided for moulds. The square-root transformation was the only one of the tested transformations that did not exhibit any correlation between the mean and the variance. As such the square-root transformation should be favoured.

Moreover, some authors have applied these models, mostly polynomial ones, to either transformed or untransformed lag phase (λ) data (Char et al., 2005; Pardo et al., 2004, 2005a,b,c,d, 2006a; Samapundo et al., 2007). Modelling of lag phase data is of interest because it allows direct prediction of the safe storage/transport periods for a given food commodity. However, modelling of lag phase data obtained under marginal growth conditions becomes difficult, because no growth situations do not allow appropriate fitting of any equation.

2.2.6. Models based on the Gamma Concept

(Zwietering et al., 1996)

Gamma Concept was proposed by Zwietering et al. (1996) and is based on the assumption that the effect of various factors affecting the growth rate of microorganisms can be combined by multiplying the separate effects. This model has the general form:

$$\gamma = \frac{\mu_{\max}}{\mu_{\text{opt}}} = \gamma(T) * \gamma(\text{pH}) * \gamma(a_w) \quad (16)$$

The relative effect of a given variable can be described by the gamma-factor of that variable:

$$\gamma(T) = \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right)^2 \quad (17)$$

$$\gamma(\text{pH}) = \frac{(\text{pH} - \text{pH}_{\min})(\text{pH}_{\max} - \text{pH})}{(\text{pH}_{\text{opt}} - \text{pH}_{\min})(\text{pH}_{\max} - \text{pH}_{\text{opt}})} \quad (18)$$

$$\gamma(a_w) = \frac{(a_w - a_{w \min})}{(1 - a_{w \min})} \quad (19)$$

The advantage of using this approach is that for every variable determining growth rate, the relative effect can be calculated, by separating the effect of the various variables (Zwietering et al., 1996). However, it needs to be noted that these models may not be applicable to a situation in which the cardinal values of one environmental factor depend on the other factors (Panagou et al., 2003). Panagou et al. (2003) combined the gamma concept with the Rosso model by using gamma factors taken from the cardinal models for a_w , pH and temperature. As cardinal models, they have the advantage that they enable easy assessment of initial parameter values and hence, facilitate the convergence procedure.

Whatever the kind of model applied, because they are empirically derived, it is very important to avoid prediction outside the domain within they were generated. Otherwise, predicted values may be highly inaccurate.

2.3. Tertiary models

Food microbiology has adopted modern methods and new concepts. Predictive models allow the microbial response in food to be predicted as a function of the food and environmental characteristics. Huge amounts of data are required to develop suitable mathematical models, making the adoption of a universal database format an urgent priority (Baranyi and Tamplin, 2004). Tertiary models could be defined as the integration of primary and secondary models in a software using databases. A database is a structured collection of information, typically stored in electronic form, for ease and speed of searching and retrieving information and security of archiving (Brul et al., 2007). There are some tertiary modelling tools; one of them is the pathogen modelling program (PMP, v.7.0) developed by the USDA—Agricultural Research Service (USDA, 2003). The PMP is an example of a tertiary model that includes great functionality, calculating pathogen growth, survival, or inactivation as a function of temperature, pH, sodium chloride, and sodium pyrophosphate concentration, with a very straightforward user interface (Marks, 2007). Other tertiary model is the latest version of ComBase (www.combase.cc) that includes a modelling tool that utilizes its database to generate growth or inactivation curves (Baranyi and Tamplin, 2004). Like PMP, ComBase tools are a collection of instruments in software to predict the microbial responses in an environment defined by two or three factors. Actually the most important program of Combase is the *ComBase predictor*, where the model is based in culture media observations. Both tertiary models, PMP and Combase, are adapted to model some pathogens bacterial growth, but none are related to either moulds growth or mycotoxin production.

3. Kinetic predictive models applied to mycotoxins production

Mould growth and mycotoxin production are associated to the presence of fungal inoculum on predisposed foods and feeds. Mycotoxins can be produced in grains of cereals, in raw materials, in different foods and feeds, in field, in transports, in stored food and in different situations in which conditions are conducive for their production. As for fungal growth, there are a lot of factors that influence the mycotoxin contamination like temperature, substrate

aeration, water activity, inoculum concentration, microbial interactions, physiological state of mould, etc., however the effects of these factors on mycotoxin production might be different from those on growth. Moreover, not all fungal growth results in mycotoxin formation and detection of fungi do not imply necessarily the presence of mycotoxin. In one hand, not all strains from a mycotoxigenic species are able to synthesize the mycotoxins, and in addition, those conditions conducive to growth may not be conducive to mycotoxin production. Finally, the absence of fungi in a food product does not necessarily imply the absence of mycotoxins. In food manufacturing, destruction of mycotoxins by conventional food processing is difficult because they are typically highly resistant and detection is complicated due to limitations in analytical methodology (Murphy et al., 2006). Thousands of mycotoxins exist, but in foods and feeds only a small number of them are of relevance. Aflatoxins, ochratoxins, patulin, fumonisins, zearalenone, and trichotecenes are of particular interest.

As said before, one approach in food safety is preventing mould growth in all the steps of the production and processing of food and thus indirectly prevent mycotoxins production. Prevention of fungal growth effectively conduces to prevention of mycotoxin accumulation. In addition, growth is a variable which presents less intraspecific variability, and the kinetics of growth are more known, thus the best alternative to prevent mycotoxin accumulation might be prediction and prevention of growth (Marín et al., 2008b). Due to the poor correlation observed between growth and mycotoxin production under different environmental conditions (Bellí et al., 2005; Marín et al., 2004; Morales et al., 2007), kinetic growth models should allow to predict the conditions under which no growth occurs. In such approach, the prediction of the growth rates through kinetic models is only relevant to determine the growth/no-growth boundary.

The second modelling approach involves direct mycotoxin analyses and modelling. From the food safety point of view the key variable to be modelled is the mycotoxin, not mould growth, however given the different abilities to synthesize mycotoxins by the different strains of a given species, extrapolation from the models obtained with one or several strains might not be representative for the majority of the strains (Marín et al., 2008a). Several other drawbacks are associated to this alternative:

- A huge number of mycotoxin analyses is required, to develop such models, involving work and economic costs.
- There is a high variability in the mycotoxin production by a given strain in a given substrate (Marín et al., 2008b). Thus mathematical modelling of mycotoxin formation may be particularly difficult.
- Although as secondary metabolites one may expect mycotoxin production to follow a curve paralleling that of growth but slightly delayed, regulation of secondary metabolism is poorly understood (Le Bars, 1988) and the relationship between the rates of primary and secondary metabolism is not clear (Pitt, 1993). Aflatoxin production generally rises during the logarithmic and deceleration phases of microbial growth (Shih and Marth, 1974), suggesting that the toxin is either a metabolite produced by growing cells or is converted biosynthetically from some other compound by growing cells. However, broader generalizations about mycotoxin formation mechanisms are difficult and the formulation of mechanistic mathematical models is therefore challenging.

3.1. Primary models

At the moment there is no widespread primary model associated to mycotoxin production. During the past decade several

publications have reported on different mycotoxins production over time either in synthetic media or food substrates. Most studies deal with ochratoxin A (OTA) and *Fusarium* toxins, due to the relevance acquired by these mycotoxins in the last few years. These studies, however, rarely take into account the possibility to model such production. OTA production on peanut, maize kernels, dried grapes and coffee beans meal extract agar medium by eight strains of *Aspergillus* section *Nigri* was evaluated at different levels of water activity (0.995–0.85), temperature (15, 25 and 30 °C) and incubation time (7, 14 and 21 days). In most cases the highest OTA levels were achieved after 7 days of incubation. The maximum OTA level was obtained at earlier growth stages when incubation temperature increased (Astoreca et al., 2007). Deoxynivalenol production by *F. culmorum* and *F. graminearum* in wheat grain over time (up to 40 days) was studied by Hope et al. (2005) and Hope and Magan (2003). Increasing concentrations ($\mu\text{g/g}$ wheat) were found with increasing incubation time, a_w , with higher levels at 25 than at 15 °C, but no models were applied. Similarly, deoxynivalenol concentration on irradiated wheat grain at a_w of 0.99 and 0.97 inoculated with *F. graminearum* at 15, 25 and 30 °C was determined after 7, 14, 21, 28, 35, 42 and 49 days of incubation (Ramirez et al., 2006). Fig. 3 shows that while at a_w of 0.97 there is an increase in deoxynivalenol (DON) over time with higher values at 25 °C, at a_w of 0.99 triggers mycotoxin accumulation resulting in high peaks and subsequent decreases which may be difficult to be fitted to any equation. Patulin was determined in *P. expansum* inoculated apples after cold storage, maintained at 20 °C for 5 days (Morales et al., 2007). The tendency of patulin was to increase with time and, except for those apples without initial lesion, it seemed to reach a peak and remain rather constant afterwards (Fig. 4). It was observed that the bigger the initial lesion after cold storage, the earlier the peak of patulin seemed to appear. Similarly, time course data for other mycotoxins (aflatoxins, citrinin, deoxynivalenol, zearalenone) production usually exhibit a rise in concentration to a peak level followed by decay in concentration to near zero (Damoglou et al., 1984; Greenhalgh et al., 1983; Koehler et al., 1985;

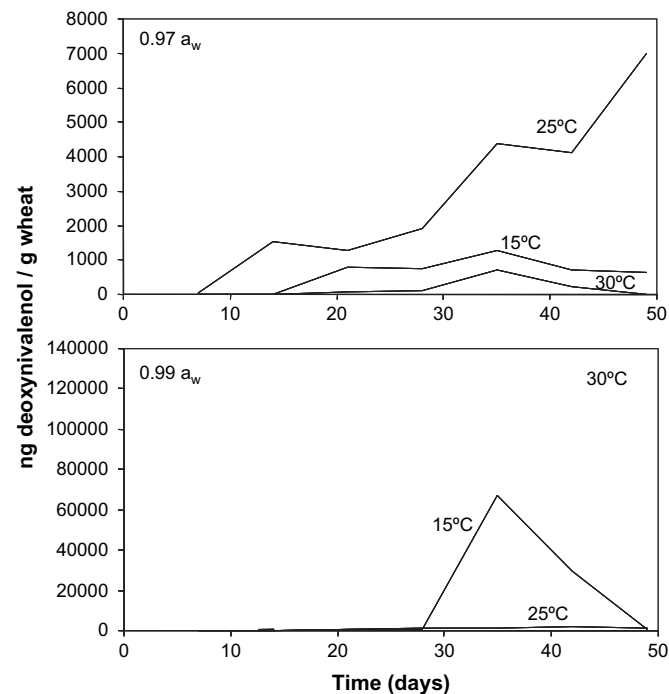


Fig. 3. Deoxynivalenol production over time in wheat as a function of temperature and water activity (from Ramirez et al., 2006).

Montani et al., 1988; Park and Bullerman, 1983; Pitt, 1993). This kind of studies quantifies the total amount of mycotoxin per weight of sample, but it is not related to the amount of mould. Thus the increasing mycotoxin concentration over time is the result of the increasing of total mould biomass, plus the increase in specific mycotoxin production (per mould weight unit), if any.

A proper mechanistic modelling should allow for estimation of the amount of mycotoxin produced per weight of fungal biomass and unit of time. Such estimated parameter would allow calculation of total mycotoxin under a given condition by multiplying g of fungal population by ng mycotoxin accumulation per g of fungal biomass per unit of time. For this reason the quantification of mycotoxin production per unit of mould is of particular interest. Some studies quantify mycotoxin production per unit of mould (either weight or surface or ergosterol content). Temporal studies (5–30 d) on OTA production in both CYA and YES by *Aspergillus carbonarius* and *Aspergillus niger* aggregate were carried out by Esteban et al. (2006a,b) at 15–30 °C and at a_w ranging from 0.86 to 0.99. They showed that *A. carbonarius* had a peak production ($\mu\text{g/g}$ agar) after 10–15 days and a decrease or maintenance afterwards, while for *A. niger* aggregate maximum production occurred at 5–10 days and decreased later. No modelling approach was included. In this case OTA was extracted from 3 plugs of agar, thus their results reflect mean specific production per g of agar, not accumulation in the total colony. As a conclusion, the mycotoxin produced per weight of fungal biomass varies over time, thus the mycotoxin produced per weight of fungal biomass and unit of time becomes a function of time. This same methodology was used in a series of studies published by Bellí et al. (2004b, 2005) and Valero et al. (2006a). This latter study included an experiment where OTA was analysed over time from points of the colony kept at constant distances from the colony centre, in this way instead of quantifying the mean OTA production per g of agar through the colony, the evolution with time of the OTA distribution through the colony was studied. They concluded that higher peak productions occurred close to the colony centre (Fig. 5).

An interesting review about a descriptive model for growth and aflatoxin formation affected by environmental conditions was presented by Pitt (1993) who developed some equations for the rates of production and degradation of aflatoxin by *A. flavus* and *A. parasiticus* as related to mould growth and environmental conditions. He assumed that the rate of toxin formation is proportional to growth rate and cell mass; in this situation each increment in new cell mass is accompanied by an increment in new toxin mass. This work is the first one and the only existing mechanistic model in the study of mycotoxin production. More recently, Molina and Giannuzzi (2002) expressed the different phases of synthesis and degradation of aflatoxin by *A. parasiticus* as a function of time by the following function:

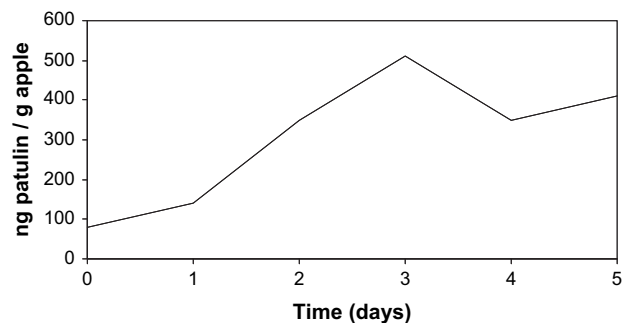


Fig. 4. Patulin production over time in cold-stored apples maintained at 20 °C for 5 days (from Morales et al., 2007).

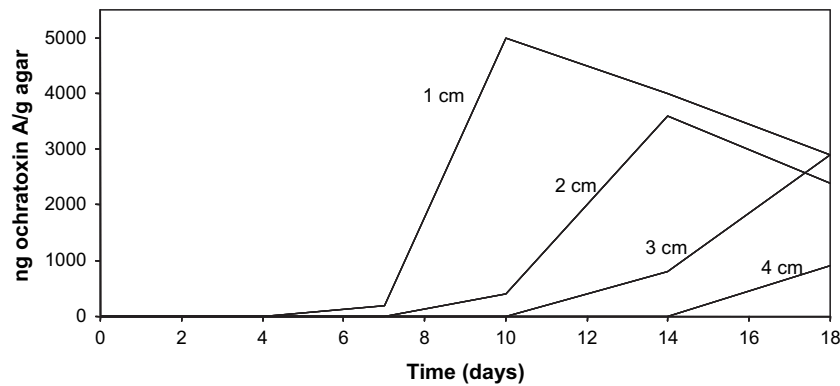


Fig. 5. Ochratoxin A accumulation by *A. carbonarius* with time at four distances (1–4 cm) from the colony centre at 0.97 a_w and 20 °C (from Valero et al., 2006a).

$$\text{Aflatoxin concentration} = C \exp \left[- \ln \left(\frac{t-a}{b} \right) \right] \quad (20)$$

where C (ppb) is the maximum aflatoxin concentration, a (h) is the time to reach the maximum aflatoxin production and b is a constant. A good data fitting to the proposed model was observed. This primary model could be used in other experimental conditions as well as for other toxins (experimental data obtained by Faraj et al., 1991) for aflatoxin production at 25 and 35 °C and for those obtained by zearalenone at 15 and 20 °C (Montani et al., 1988).

3.2. Secondary models

Few are the studies presenting secondary model equations for mycotoxins production. Some works presented toxin formation under different environmental conditions, but at certain time periods, thus no primary models were applied. Polynomial models are used to present the effect of a_w and temperature mainly at a given time (Marín et al., 1999; Pardo et al., 2004).

Bellí et al. (2004b) determined the temporal (5–20 days) OTA production profile of *Aspergillus* section *Nigri* isolated from grapes at different a_w levels. Results were modelled by a multiple linear regression and response surface predictive models were obtained. Maximum amounts of OTA were found at the earlier growth stages (5 days for *A. carbonarius* and 7–13 days for *A. niger* aggregate). The amounts of OTA detected decreased with time, being minimal after 20 days of incubation. Latter, Marín et al. (2006a) studied the kinetics of OTA production and accumulation by *A. carbonarius* on a synthetic grape medium at different temperature levels (7–42 °C). OTA production was followed for 10 days, and it was observed that at higher temperature levels OTA was produced after only 2–4 days, while at temperatures below 25 °C, maximum OTA producing capacity was detected later, after 10 days. OTA accumulation (μg OTA/colony) was modelled using the sigmoidal Gompertz model. This variable includes both increase in colony diameter and variation of specific OTA production per unit if colony surface. Besides the Gompertz model, any other sigmoidal model could have been used. The main challenge is the production of repeatable and reproducible data, as there is a well-known intrinsic biological variability in mycotoxins accumulation (Marín et al., 2006a).

The effect of temperature on aflatoxin (B_1 , B_2 , G_1 and G_2) production in agar media at different pH (5.5 and 5.9) was studied. The effect of temperature on aflatoxin production at different pH levels was analysed using an Arrhenius-like temperature function that was developed with flexible parameters, as developed by Pitt (1993):

$$f_T = a \exp \left\{ - \left[\frac{\alpha^2}{T - T_{\min t}} + \frac{\alpha^2}{T_{\max t} - T} \right] \right\} \quad (21)$$

where f_T = relative toxin formation $C_T/C_{T_{\max t}}$ experimental, $T_{\min t}$ = minimum temperature for toxigenesis in °C, $T_{\max t}$ = maximum temperature for toxigenesis in °C, α = shape parameter, and a = scaling parameter.

Finally, Baert et al. (2007) modelled patulin accumulation in an apple-based medium and on apples as a function of temperature and %O₂ during the cold storage time. They applied the following polynomial equation:

$$\begin{aligned} \text{patulin concentration (mg/kg)} = & a.T + b.S + c.O + d.T.S \\ & + e.T.O + f.S.O + g.T.S.O \\ & + h.T^2 + i.T^2.S + j.T^2.O \\ & + k.T^2.S.O \end{aligned} \quad (22)$$

with S = surface (cm^2) and O = oxygen level (%)

In this case mould growth (measured as surface of decayed apple) was included in the model as a factor.

Germination, growth and mycotoxin production models applied to moulds have included factors such as water activity and temperature, mainly. Some others include pH and preservative concentrations. In food products, nutrients are not usually a limitation, and they are not considered in models related to food safety. Obviously, many other variables are neglected, initial inoculum size and accompanying microbiota, among them. While the latter might be quite difficult to be taken into consideration, as it has been shown that presence of competing microbiota may lead to both stimulation and inhibition of mould growth/mycotoxin production depending on environmental factors (Marín et al., 1998a; Valero et al., 2006b; Velluti et al., 2000), an effort should be made to assess the effects of different inoculum levels on the developed models and to standardise the initial inoculum in designed experiments. Baert et al. (2008), working with *P. expansum* germination and growth in apples concluded that the inoculum size influences the estimated growth parameters and should be considered in quantitative risk assessments and for the design of challenge tests and experiments to gather data for predictive growth models.

Finally, these models are developed assuming constant levels of factors, however, factors such as temperature are variable during transport and storage of many food commodities. Tertiary models could be used to take into account this temperature variability by linking the different models at the successive temperature levels. Some authors, however, have pointed out that alternating different

temperature regimes may stimulate growth and mycotoxins production under certain circumstances (Palacios-Cabrera et al., 2004). In practice, foods are subject to fluctuating temperatures and models must predict accurately for all situations (Neumeier et al., 1997).

4. Probabilistic models applied to mould growth and mycotoxins production

Predictive models in food microbiology can be divided, according to their aim, into two main categories: kinetic models (described till here in this review) and probability models. Kinetic models that predict growth of food-borne fungi are effective under a wide range of conditions; however, they are less useful close to the boundary between the growth and no growth. Probabilistic models are useful where the objective is to determine whether or not microbial growth can occur under specific conditions. In many situations it is important to ensure that microorganisms do not contaminate foods. Consequently, probability modelling is particularly useful when pathogenic or mycotoxin-producing species are involved. Probability models allow the prediction of whether a particular event, such as growth or toxin production, might occur, under various conditions (Gibson and Hocking, 1997). Probabilistic growth models are built from the proportion of “growth/no growth” responses throughout the experimental design space at a defined point in time (Brul et al., 2007).

Logistic regression is a useful tool for modelling the boundary between growth and no growth. A logistic regression model relates the probability of occurrence of an event, Y , conditional on a vector, x , of explanatory variables (Hosmer and Lemeshow, 1989). The specific model of the logistic regression is as follows:

$$P(x) = \frac{\exp(\sum b_i x_i)}{1 + \exp(\sum b_i x_i)} \quad (23)$$

where x_i are factor variables (T , a_w , pH, ...) and b_i are constants to be estimated.

The logit transformation of $P(x)$ is defined as:

$$\text{logit}(P) = \ln \frac{P(x)}{1 - P(x)} = \sum b_i x_i \quad (24)$$

Few studies report on the use of probability models for prediction of moulds growth or mycotoxins production. The first one dates from 2001, when logistic regression was used to create predictive models to predict the probability of growth of spoilage moulds (*A. niger* and *Penicillium spinulosum*) in response to various preservative systems in ready to drink beverages at different levels of pH, titratable acidity and sugar content (Battey et al., 2001). The boundary between growth and non growth of *Eurotium chevalieri* in milk jam as a function of a_w , pH, potassium sorbate and storage time was predicted by means of the probabilistic model using logistic regression, which provided a wide range of formulation possibilities depending on the targeted shelf-life (Char et al., 2005). The same methodology has been recently applied by Marín et al. (2008b) to develop suitable validated models to predict the growth and OTA production boundaries by an *A. carbonarius* isolated from pistachios as a function of moisture content and storage temperature of pistachios. Probability models were applied in this work to mycotoxin accumulation for the first time. Finally, probability models for prediction of growth of aflatoxigenic moulds in powdered *Capsicum* fruits as a function of its water availability were developed on 3% chilli powder extract agar at different water activity levels. Linear logistic regression was also applied to predict the probability of growth over storage time. Moisture content levels

required for safe production, storage and transport of chillies and chilli powder were predicted (Marín et al., 2009).

Storage trial cereal samples in which growth of *P. verrucosum* was observed were assigned values of either 0 or 1, depending on whether or not the levels of OTA exceeded the legislative limit of 5 µg/kg grain. Logistic regression was used to calculate the probability that the legislative limit is exceeded, given a certain number of *P. verrucosum* colonies per gram of grain and storage conditions (water activity and temperature) (Lindblad et al., 2004).

5. Validation of predictive models

Predictive food microbiology is based upon the premise that the responses of populations of microorganisms to environmental factors are reproducible and that it is possible, from past observations, to predict the responses of microorganisms in a particular environment (Ross, 1996). Validation is an essential step after modelling. The first stage of validation, when proposing a new type of model is often internal validation (te Giffel and Zwietering, 1999) which means validation is performed on the same data used for building the model (Ratkowsky and Ross, 1995). However, further external validation, using new data not used for fitting the model, would appear to be essential to confirm the robustness of the model (Delignette-Muller et al., 1994). Besides, predictive models are often built on data obtained in laboratory medium. Extrapolation to predictions in food products is not straightforward (Dalgaard and Jørgensen, 1998; McClure et al., 1993) because of the complexity of these media. Predictive models are often built under laboratory conditions with synthetic media and take a limited number of factors into account compared to the numerous factors influencing growth moulds in food products (Pinon et al., 2004). Therefore, a good way of validating a model is to compare its prediction to data obtained for food products. Models cannot be used with confidence until such a comparison is made and hence validation is an essential step enabling researchers to understand the applicable range of models and also limits of their performance (Jagannath and Tsuchido, 2003).

Three levels are thus considered in validation of food predictive models. In any case validation involves the comparison between observed (validation set of samples) and predicted values through calibration model. Graphic validation may be done just plotting observed values versus predicted ones, which should be as close as possible to the target line (straight line at a 45° angle with positive x axis) for a model with good prediction performance.

For numerical validation, the indices proposed by Ross (1996) may be used. The indices (bias factor and accuracy factor) assess the level of confidence one can have in the predictions of the model and whether the model displays any bias which could lead to ‘fail-dangerous’ predictions.

The bias and accuracy factors provide an objective summary of the performance of predictive models in food microbiology. They are insufficient on their own because the bias factor, as an average, may obscure systematic deviations between predicted and observed responses in one part of the response surface if they are ‘balanced’ by deviations in another part of the response surface. Such behaviour might be signalled by a larger accuracy factor, but it is still important to plot the predicted and observed values to guard against such systematic deviations. Nonetheless, though imperfect, the bias and accuracy factors are suggested as a first step towards the development of an objective and useful definition of the term ‘validated model’ (Ross, 1996).

Very few studies in scientific literature have externally validated the models developed to describe fungal growth, and they rarely present accuracy and bias factors. For example, Battey et al. (2001) constructed a probability model for *A. niger* and *P. spinulosum* on

a beverage analogue and validated it in an external set of beverage analogues, too. Samapundo et al. (2007) modelled *Aspergillus flavus* and *Aspergillus parasiticus* growth on irradiated maize grain, and performed the numerical validation on an external independent set of experiments on irradiated maize grain, too. Baert et al. (2007) developed a series of models for *Penicillium expansum* growth in apple puree agar medium, which were validated in apples. Marín et al. (2008b) validated the probability growth and OTA models developed for *A. carbonarius* on pistachio nuts in an independent set of experiments prepared on pistachio nuts.

6. Conclusion

The presence of mycotoxins in food products is a chemical hazard of biological origin of increasing concern due to the wide range of food types where they can be found. As for predictive microbiology, predictive mycology should become a useful tool in HACCP plans in the food production and processing; however, some limitations intrinsic to moulds characteristics have delayed its development. Most publications on predictive mycology have just come up during the last decade, including both kinetic and probability models. Some primary and secondary kinetic models have to date been applied to germination and growth of various mycotoxigenic fungi. Prevention of fungal growth may be one way to prevent mycotoxin accumulation. Regarding mycotoxins, little progress has been made in their kinetic modelling to date. Probability models might be an additional alternative which allows prediction of probability of mycotoxin presence. Prediction of a certain mycotoxin level, such as the legal limit, may be particularly difficult because mycotoxin level is mostly a function of the contaminating fungal strain apart from the environmental conditions which may be included in a model. Finally, validation of models in food products is crucial for development of suitable models and their direct application to food safety management.

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