International Journal of Food Microbiology 156 (2012) 60–67

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Modelling the effect of temperature and water activity of *Aspergillus flavus* isolates from corn

A. Astoreca [a,*], G. Vaamonde [a], A. Dalcero [b], A.J. Ramos [c], S. Marín [c]

[a] Laboratorio de Microbiología de Alimentos, Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina
[b] Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, UNRC, Río Cuarto, Argentina
[c] Food Technology Department, Lleida University, UTPV-XaRTA-CRA, Lleida, Spain

ARTICLE INFO

Article history:
Received 7 November 2011
Received in revised form 14 February 2012
Accepted 1 March 2012
Available online 8 March 2012

Keywords:
Aspergillus flavus
Kinetic model
Probability model
a_w
Temperature

ABSTRACT

The aim of this study was to model the effects of temperature (10–40 °C) and a_w (0.80–0.98), in two media (Czapek yeast agar: CYA; corn extract medium: CEM) on the growth rates and growth boundaries (growth–no growth interface) of three strains of *A. flavus* isolated from corn in Argentina. Both kinetic and probability models were applied to colony growth data. The growth rates obtained in CYA were significantly ($p<0.05$) greater than those obtained in CEM medium. No significant differences ($p<0.05$) were observed among the three isolates. The growth rate data showed a good fit to the Rosso cardinal models combined with the gamma-concept with $R^2 = 0.98–0.99$ and RMSE = 0.60–0.78, depending on media and isolates. The probability model allowed prediction of safe storage (p of growth <0.01) for one month for moist maize (e.g. 0.90 a_w) provided temperature is under 15 °C, or for dry maize (e.g. 0.80 a_w) provided temperature is under 27 °C. Storage at <0.77 a_w would be safe regardless of the storage temperature. Probability models allow evaluation of the risk of fungal contamination in the process of storage, so the results obtained in this study may be useful for application in systems of food safety management.

1. Introduction

Fungal spoilage of food and feedstuffs is a worldwide problem because it causes large economic losses in the producing countries and a serious risk of undermining public health under inappropiate storage conditions. Harvested grains can be colonised by several fungal genera, including various species of *Aspergillus*, leading to deterioration and mycotoxin production. Attention is continuously focused on corn (*Zea mays L.*) because it is one of the most important dietary staple foods in the world (FAO, 2002). This crop can be contaminated by a variety of toxigenic mould species during pre and postharvest periods, *Aspergillus flavus* being the dominant species in this respect (Donner et al., 2009; Jaime-Garcia and Cotty, 2010). *A. flavus* is the main producer of aflatoxins (AFs), which include the most potent natural carcinogen known (JECFA, 1997). Some isolates of this species are able to produce other mycotoxins, particularly cyclopiazonic acid (CPA), which is toxic to a variety of animals and has been implicated in human poisoning (Dorner et al., 1983; Rao and Husain, 1985). Studies of naturally occurring aflatoxins in corn have demonstrated that the levels of contamination are variable (Nesci and Etcheverry, 2002). Information on natural CPA contamination is scarce (Astoreca et al., 2011). Prevention of *A. flavus* growth is needed to diminish the risk of contamination with these toxic secondary metabolites.

The growth of moulds and mycotoxin accumulation in foods and feeds depend on the effect of multiple variables such as a_w, temperature, pH, atmosphere composition, substrate, interaction between species, time, etc. To date several studies on the effect of biotic and abiotic factors on growth and aflatoxin production by toxigenic species have been published (Gibson et al., 1994; Holmquist et al., 1983; Lacey et al., 1991; Marín et al., 1998; Niles et al., 1985; Pitt and Miscamble, 1995; Sautour et al., 2001, 2002; Trenk and Hartman, 1970). Generally, a_w and temperature are regarded as the main controlling factors determining the potential for growth (Dantigny et al., 2005; Panagou et al., 2003; Plaza et al., 2003).

In order to improve the quality and safety of food, there is a need for tools allowing the prediction of fungal growth (Dantigny et al., 2005). Growth of a fungal colony is not synonymous with production of mycotoxins, even for a mycotoxigenic mould, because the environmental conditions may allow growth but not production of mycotoxins and toxin production may be triggered under stressing conditions for growth. Prevention of fungal growth effectively prevents mycotoxin accumulation (García et al., 2009). Mathematical modelling can be a useful tool to predict and, consequently, to prevent the growth of mycotoxigenic moulds. A few studies have applied secondary kinetic models to model growth of aflatoxigenic *A. flavus*

* Corresponding author at: Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Intendente Güiraldes 2160, Pabellón II, Ciudad Universitaria (C1428EGA), Buenos Aires, Argentina. Tel./fax: +54 11 4576 3346.
E-mail address: andreaftorca@hotmail.com (A. Astoreca).

0168-1605/$ – see front matter.
doi:10.1016/j.ijfoodmicro.2012.03.002

(Baranyi et al., 1997; Gibson et al., 1994; Marín et al., 2009; Samapundo et al., 2007a; Sautour et al., 2001; Yue et al., 2011) in a number of substrates, while probability models have seldom been used, as far as we know. Whereas Gibson et al. (1994) and Sautour et al. (2001) proposed pioneer kinetic models for the modelling of mould growth as affected by a_w, only Samapundo et al. (2007a) and Yue et al. (2011) considered the combined effect of both a_w and temperature in their models. Sautour et al. (2001) developed a temperature type (Rosso type) model to describe the relationship between the growth of several fungi (including *A. flavus*) on potato dextrose agar and a_w, from which cardinal a_w (a_{wmin}, a_{wopt} and a_{wmax}) could be estimated. This model was later applied by Marín et al. (2009) for the prediction of growth of aflatoxigenic moulds isolated from red chili powder. Linear logistic regression was also applied by the latter to predict the probability of growth over storage time. Similarly, García et al. (2011) developed a probability modelling approach that could be satisfactorily employed to quantify the combined effect of temperature and a_w on the growth responses of *Aspergillus parasiticus* (isolated from peanuts).

The objective of the present study was to use the gamma concept to assess the combined effects of temperature and a_w on the growth rate of three strains of *A. flavus* isolated from corn in Argentina based on data obtained in two different media. However this approach did not account for the interactions between temperature and a_w close to the growth/no growth interface. Accordingly, a probabilistic approach was used to model the growth boundaries.

2. Materials and methods

2.1. Experimental design

A full factorial design was used in which four factors were assayed: isolate, media, a_w and temperature. Two growth parameters (μ and λ) were recorded at each condition as response variables. The a_w levels assayed were 0.80, 0.83, 0.86, 0.90, 0.94, 0.96 and 0.98 and the incubation temperatures were 10, 15, 25, 30, 35 and 40 °C. Four replicates for each treatment were used.

2.2. Fungal isolates

Three strains of *A. flavus* isolated from corn used in the production of poultry feeds in Argentina were used in this study. The corn samples were collected from a feed plant located in the south of Córdoba Province in 2009 and these isolates are kept in the Buenos Aires Faculty of Sciences (BAFC) collection. They had previously been characterised to be aflatoxin and/or CPA producers: BAFC4273 (AFB_1−/CPA+), BAFC4274 (AFB_1+/CPA+) and BAFC4275 (AFB_1+/CPA−) (Astoreca et al., 2011).

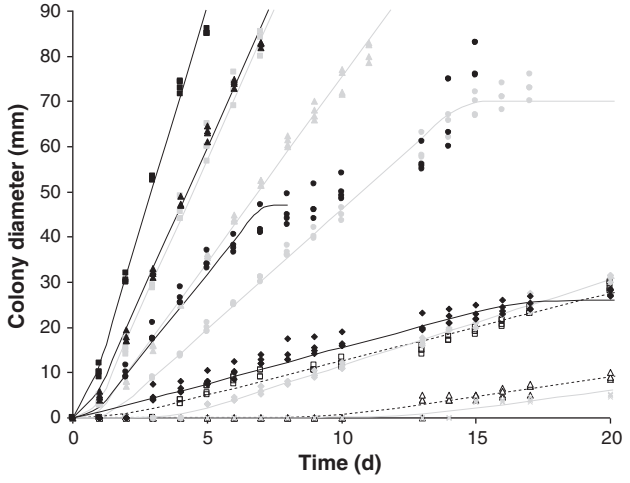

Fig. 1. Growth curves for *A. flavus* BAFC4273 on CYA at 35 °C (■, 0.98 a_w; ▲, 0.94 a_w; ●, 0.90 a_w; ◆, 0.86 a_w), 25 °C (▩, 0.98 a_w; ▲, 0.94 a_w; ●, 0.90 a_w; ◆, 0.86 a_w; ×, 0.83 a_w) and 15 °C (□, 0.98 a_w; △, 0.94 a_w) fitted to Baranyi model (—, 35 °C; —, 25 °C; —, 15 °C).

2.3. Media

Czapek Yeast Agar (CYA) and corn extract medium (CEM) were used in this study. The later was made by boiling 30 g of corn in 1 L of distilled water for 45 min and filtering the resulting mixture through a double layer of muslin. The volume was made up to 1 L and 1.5% agar was added. The a_w of both media was modified by the addition of known amounts of glycerol to reach 0.80, 0.83, 0.86, 0.90, 0.94, 0.96 and 0.98. The a_w of representative samples of each medium was checked with an AquaLab Series 3 (Decagon Devices, Inc., WA, USA). Additionally, control plates were prepared and measured at the end of the experiment in order to detect any significant deviation of a_w.

2.4. Inoculation and incubation conditions

The media for each treatment were centrally inoculated using 5 μl of a fungal spore suspension harvested from 7-day-old cultures on malt extract agar (MEA) using glycerol solutions adjusted to the a_w appropriate for each treatment. The suspensions were mixed and diluted to obtain a suspension of 10^5 spores/ml adjusted using a Thoma chamber. Inoculated Petri dishes of the same a_w were sealed in polyethylene bags. Four replicate plates per treatment were used and incubated for 28 days at 10, 15, 25, 30, 35 and 40 °C.

2.5. Growth assessment

Two perpendicular diameters of the growing colonies were measured daily (mm) until the colony reached the edge of the plate. The diameters (D) of the colonies were plotted against time and a non linear regression was applied to estimate the maximum growth rate (μ_{max}, mm/day), time to visible growth (λ, day) and maximum colony diameter (D_{max}), if applicable, by fitting the experimental data to the primary model of Baranyi and Roberts (1994) [1] by using Statgraphics® Plus version 5.1 (Manugistics, Inc, Maryland, USA).

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

Analysis of variance (ANOVA) was applied to μ_{max} and λ repeated data in order to establish the significance of the assayed factors (a_w, temperature, strain, medium) The estimates of μ_{max} were further fitted to the gamma-concept (Zwietering et al., 1996) combined secondary models proposed by Rosso et al. (1995) and Sautour et al. (2001) by multivariable regression to describe the effect of temperature and a_w on fungal growth rate. The model is described by the following equation:

$$\mu_{max}(T, a_w) = \mu_{opt} \cdot \tau(T) \cdot \rho(a_w) \quad (2)$$

where

$$\tau(T) = \left(\frac{(T-T_{min})^2 \cdot (T-T_{max})}{(T_{opt}-T_{min}) \cdot \left[(T_{opt}-T_{min})(T-T_{opt}) - (T_{opt}-T_{max})(T_{opt}+T_{min}-2T)\right]}\right) \quad (3)$$

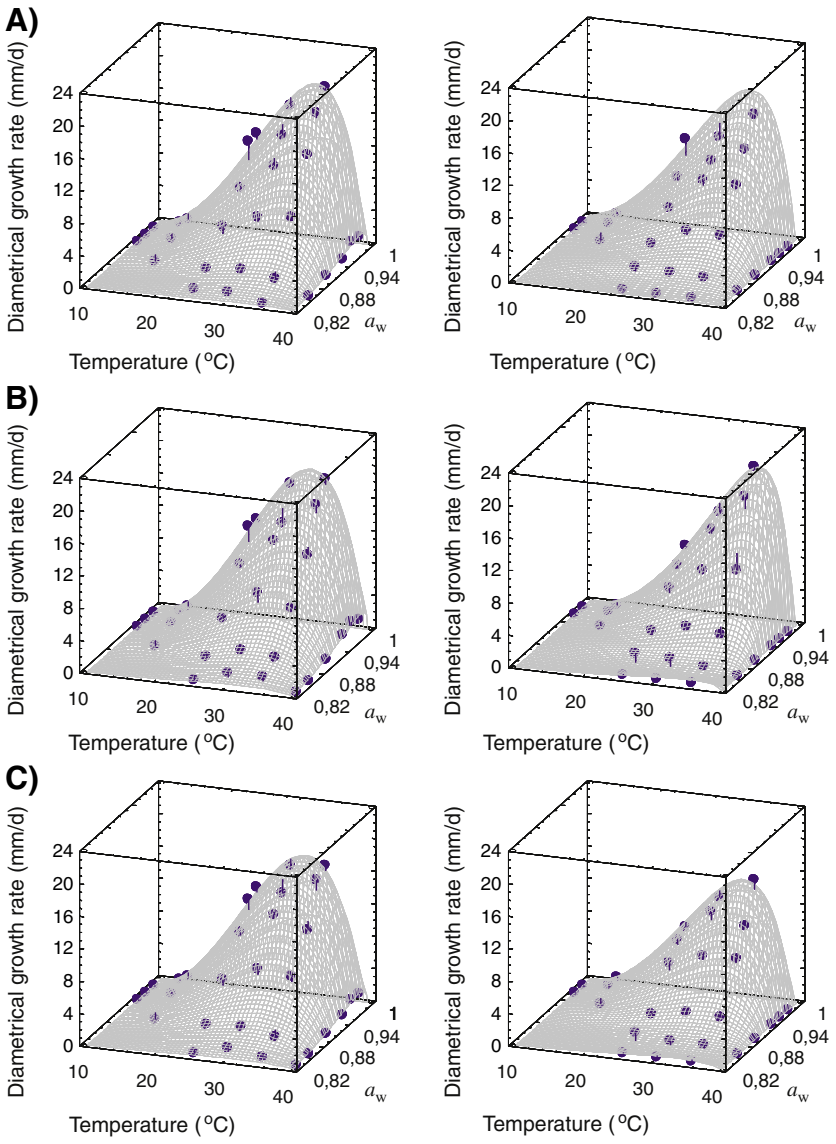

Fig. 2. Multifactorial cardinal model (lines) fitted to observed growth rate values (symbols) of *A. flavus* isolates: A) BAFC4273, B) BAFC4274 and C) BAFC4275 in CYA (left) and CEM (right) media at different a_w and temperature levels. a_w levels: 0.83 (♦)- 0.86 (×)- 0.90 (◊)- 0.94 (▲)- 0.96 (△) and 0.98 (■).

$$\rho(a_w) = \left(\frac{(a_w - a_{w\min})^2 \cdot (a_w - a_{w\max})}{(a_{w\,opt} - a_{w\min}) \cdot \left[(a_{w\,opt} - a_{w\min})(a_w - a_{w\,opt}) - (a_{w\,opt} - a_{w\max})(a_{w\,opt} + a_{w\min} - 2a_w)\right]}\right) \quad (4)$$

where

T_{min}	is the temperature below which growth is no longer observed
T_{max}	is the temperature above which no growth occurs
T_{opt}	is the temperature at which maximum growth rate equals its optimal value μ_{opt}
a_{wmin}	is the a_w below which growth is no longer observed
a_{wmax}	is the a_w above which no growth occurs
a_{wopt}	is the a_w at which maximum growth rate equals its optimal value μ_{opt}

The non linear regression option in Statgraphics® Plus version 5.1 (Manugistics, Inc, Maryland, USA) was used to fit the multifactorial secondary models to the data. Homogeneity of variance of the untransformed dependent variable was checked by determining the correlation between the mean growth rate and the variance of the three isolates at different temperature and a_w levels. Untransformed μ_{max} data were uncorrelated to the variance, and for this reason Eq. (2) was used with no further transformation of growth rate data.

2.6. Modelling of the growth/no growth interface

For each treatment of the three fungal isolates, growth data were converted into probabilities of growth by assigning the value of 1 in the case where visible fungal growth was evident, and 0 in the case of absence of growth during the overall period of the experiment. The resulting data were fitted to a logistic regression model as previously described (Ratkowsky and Ross, 1995) to determine the growth/no growth boundaries regarding a_w and temperature. The model employed was a full second order logistic regression model (Battey et al., 2002) that included also linear and quadratic terms for time [5]

$$Logit\ P = ln\left(\frac{P}{1-P}\right) = b_0 + b_1 a_w + b_2 T + b_{11} a_w^2 + b_{22} T^2 + b_{12} a_w T + time + time^2 \quad (5)$$

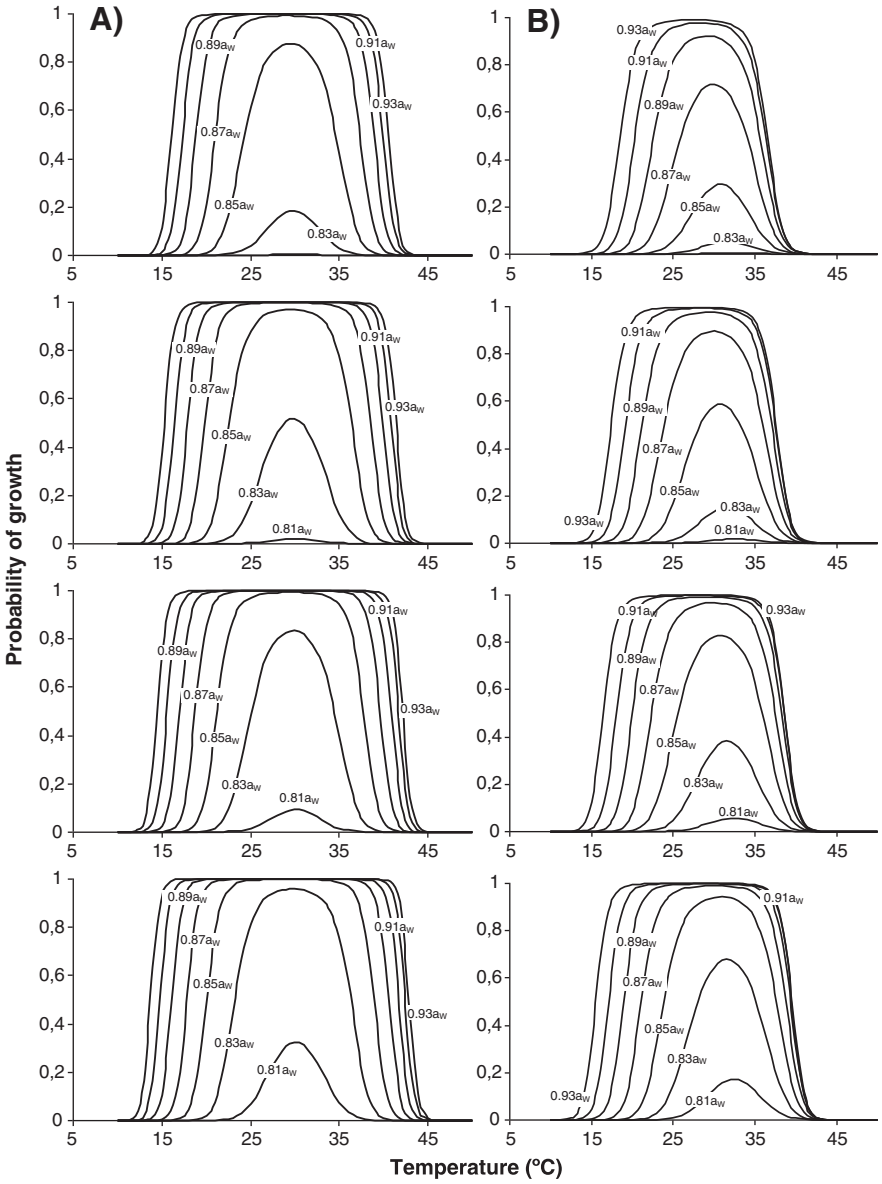

Fig. 3. The predicted effect of temperature and a_w on probability of growth of *A. flavus* BAFC4273 isolate growing in A) CYA and B) CEM incubated for 7, 14, 21 and 28 days.

Where P is probability that growth occurs and b_i are the coefficients to be estimated. The equation was fitted by using Statgraphics® Plus version 5.1 (Manugistics, Inc, Maryland, USA) linear logistic regression procedure. The automatic variable selection option with a backward stepwise factor selection method was used to choose the significant effects ($P<0.05$). The cut off level for growth/no-growth was set at $P=0.5$.

3. Results

3.1. Kinetic primary model

Increasing diameters of the three isolates in both media followed, in general, a lag-linear curve with some exceptions occurring mainly at 30–35 °C and 0.83–0.90 a_w where growth followed a sigmoidal function (with an upper asymptote) (Fig. 1). Maximum growth rate (μ_{max}) and time to visible growth (λ) were estimated through Baranyi's primary model (Tables 1 and 2). The differences in μ_{max} and λ among the three assayed isolates were not significant ($p>0.05$). For the three assayed moulds, μ_{max} decreased and λ increased under marginal conditions. No growth was observed under extreme conditions but these were slightly different depending on the culture media ($p<0.05$). In CYA, no growth was observed at 10 °C at any assayed a_w, nor at 15 °C at a_w <0.94 and at 40 °C at a_w <0.90; growth was not observed at 0.80 a_w. In CEM, the growth was more limited since none of the isolates were able to grow at 10 °C and 40 °C regardless of the a_w level, while they only grew at 15 °C at a_w >0.94, and growth was never observed at 0.83 a_w. Overall, growth was faster in CYA than in CEM ($p<0.05$).

3.2. Secondary modelling for the effects of a_w and temperature on the growth rate and time to visible growth

The cardinal values of environmental factors (minimum, maximum and optimum value) estimated with the cardinal secondary model are shown in Table 3 and the fitted models are presented in Fig. 2. Model [2] showed a good fit with $R^2=0.983–0.988$ and RMSE = 0.600–0.782, depending on medium and isolates. The three isolates showed a similar pattern of behaviour, based on both, the estimated values of the secondary model and the results obtained experimentally. In the temperature and a_w range studied, the optimal conditions of growth for the three assayed *A. flavus* isolates on both

Table 1
Mean (n = 4) estimated maximum growth rates (μ_{max}) and time to visible growth (λ) for *Aspergillus flavus* isolates on Czapek Yeast Agar (CYA) at different temperature and water activity levels.

Temperature (°C)	a_W	BAFC4273		BAFC4274		BAFC4275	
		μmax(mm/day) ± SD	λ(day) ± SD	μmax(mm/day) ± SD	λ(day) ± SD	μmax(mm/day) ± SD	λ(day) ± SD
15	0.83	–	–	–	–	–	–
	0.86	–	–	–	–	–	–
	0.90	–	–	–	–	–	–
	0.94	0.95 ± 0.15^{a}	10.03 ± 0.83^{a}	1.02 ± 0.08^{a}	10.74 ± 0.67^{a}	1.29 ± 0.11^{a}	7.86 ± 0.34^{a}
	0.96	1.98 ± 0.05^{b}	3.66 ± 0.47^{b}	1.71 ± 0.15^{c}	3.07 ± 0.40^{b}	2.11 ± 0.06^{c}	3.39 ± 0.34^{b}
	0.98	1.50 ± 0.03^{ab}	1.72 ± 0.24^{c}	1.29 ± 0.08^{b}	1.85 ± 0.72^{c}	1.60 ± 0.02^{b}	1.88 ± 0.21^{c}
25	0.83	0.76 ± 0.05^{a}	12.39 ± 1.14^{a}	–	–	-	-
	0.86	1.91 ± 0.06^{b}	3.89 ± 0.22^{b}	1.53 ± 0.06^{a}	2.77 ± 0.08^{a}	2.13 ± 0.16^{a}	3.86 ± 0.09^{a}
	0.90	5.29 ± 0.31^{c}	1.34 ± 1.13^{c}	4.34 ± 0.57^{b}	0.70 ± 0.39^{b}	5.88 ± 0.41^{b}	1.80 ± 0.33^{b}
	0.94	8.20 ± 0.32^{d}	0.82 ± 0.16^{d}	9.22 ± 0.07^{c}	0.88 ± 0.06^{b}	9.86 ± 0.21^{c}	1.12 ± 0.12^{c}
	0.96	12.94 ± 0.21^{e}	0.86 ± 0.09^{d}	13.00 ± 0.35^{d}	0.91 ± 0.09^{b}	12.99 ± 0.29^{d}	1.00 ± 0.10^{cd}
	0.98	13.16 ± 0.63^{e}	0.67 ± 0.19^{d}	12.97 ± 0.51^{d}	0.65 ± 0.09^{b}	13.64 ± 0.64^{d}	0.72 ± 0.09^{d}
30	0.83	0.81 ± 0.26^{a}	7.81 ± 2.85^{a}	1.34 ± 0.49^{a}	6.07 ± 0.68^{a}	1.16 ± 0.27^{a}	7.74 ± 1.66^{a}
	0.86	2.24 ± 0.59^{b}	2.09 ± 1.51^{b}	2.83 ± 0.46^{b}	1.09 ± 0.16^{b}	2.41 ± 0.11^{b}	1.68 ± 0.14^{b}
	0.90	6.91 ± 0.12^{c}	0.66 ± 0.05^{b}	7.99 ± 0.25^{c}	0.73 ± 0.14^{bc}	7.70 ± 0.21^{c}	0.93 ± 0.08^{b}
	0.94	11.33 ± 0.06^{d}	0.57 ± 0.03^{b}	12.63 ± 0.03^{d}	0.71 ± 0.04^{bc}	12.58 ± 0.14^{d}	0.78 ± 0.05^{b}
	0.96	14.32 ± 0.06^{e}	0.39 ± 0.04^{b}	13.92 ± 0.11^{e}	0.27 ± 0.05^{c}	14.21 ± 0.07^{e}	0.45 ± 0.03^{b}
	0.98	17.02 ± 0.24^{f}	0.34 ± 0.06^{b}	17.92 ± 0.46^{f}	0.36 ± 0.08^{c}	16.71 ± 0.18^{f}	0.32 ± 0.06^{b}
35	0.83	–	–	1.31 ± 0.53^{a}	5.25 ± 1.17^{a}	0.48 ± 1.14^{a}	4.73 ± 3.99^{a}
	0.86	1.60 ± 0.41^{a}	0.33 ± 0.19^{a}	2.41 ± 0.45^{b}	0.56 ± 0.23^{b}	1.64 ± 0.40^{b}	0.65 ± 0.47^{b}
	0.90	7.36 ± 0.33^{b}	0.66 ± 0.16^{b}	6.63 ± 0.29^{c}	0.30 ± 0.07^{b}	7.28 ± 0.36^{c}	0.68 ± 0.01^{b}
	0.94	13.26 ± 0.38^{c}	0.54 ± 0.05^{ab}	11.32 ± 0.78^{d}	0.27 ± 0.09^{b}	11.14 ± 0.68^{d}	0.14 ± 0.11^{b}
	0.96	17.56 ± 1.16^{d}	0.37 ± 0.05^{a}	16.59 ± 0.06^{e}	0.27 ± 0.01^{b}	16.46 ± 0.27^{e}	0.32 ± 0.04^{b}
	0.98	19.83 ± 1.22^{d}	0.42 ± 0.11^{a}	18.90 ± 0.28^{f}	0.33 ± 0.1^{b}	17.21 ± 0.70^{e}	0.39 ± 0.03^{b}
40	0.83	–	–	–	–	–	–
	0.86	–	–	–	–	–	–
	0.90	0.70 ± 0.04^{a}	14.23 ± 2.11^{a}	0.83 ± 0.06^{a}	12.63 ± 2.74^{a}	0.73 ± 0.09^{a}	11.50 ± 5.83^{a}
	0.94	0.94 ± 0.02^{b}	10.41 ± 2.72^{a}	2.00 ± 0.59^{b}	3.94 ± 1.59^{b}	1.08 ± 0.14^{b}	2.74 ± 0.38^{b}
	0.96	2.15 ± 0.16^{d}	4.01 ± 1.49^{b}	2.70 ± 0.24^{c}	2.58 ± 0.96^{b}	2.18 ± 0.24^{c}	3.74 ± 0.44^{b}
	0.98	1.82 ± 0.03^{c}	5.73 ± 1.84^{b}	2.09 ± 0.03^{bc}	3.27 ± 1.64^{b}	1.93 ± 0.05^{c}	4.95 ± 2.62^{b}

No growth was observed for 28 days. SD: standard deviation.
For each strain and temperature level, different letters next to the means mean significant differences ($p<0.05$) among growth at the different a_w levels according to Tukey HSD test.

tested media were 0.98–1 a_w and 32–36 °C with maximum predicted growth rates of 18.3–20.0 mm/day and 15.1–19.6 mm/day on CYA and CEM, respectively. Estimated a_{wmin} varied from 0.79 to 0.85; all estimations may well be accurate, as a_{wmin} values were 0.01–0.05 units under the a_w where growth was observed. Regarding temperature, T_{min} predicted values in model [2] were between 8.4 and 10.0 °C,

Table 2
Mean (n = 4) estimated maximum growth rates (μ_{max}) and time to visible growth (λ) for *Aspergillus flavus* isolates on CEM at different temperature and water activity levels.

Temperature (°C)	a_W	BAFC4273		BAFC4274		BAFC4275	
		μmax(mm/day) ± SD	λ(day) ± SD	μmax(mm/day) ± SD	λ(day) ± SD	μmax(mm/day) ± SD	λ(day) ± SD
15	0.83	–	–	–	–	–	–
	0.86	–	–	–	–	–	–
	0.90	–	–	–	–	–	–
	0.94	–	–	–	–	–	–
	0.96	1.30 ± 0.28^{a}	3.58 ± 1.99^{a}	0.86 ± 0.08^{a}	4.36 ± 1.74^{a}	1.45 ± 0.08^{a}	3.07 ± 0.64^{a}
	0.98	1.48 ± 0.14^{a}	2.87 ± 0.43^{a}	0.75 ± 0.04^{b}	1.57 ± 0.29^{b}	1.54 ± 0.10^{a}	3.06 ± 0.54^{a}
25	0.83	–	–	–	–	–	–
	0.86	1.43 ± 0.26^{a}	8.29 ± 1.73^{a}	1.31 ± 0.07^{a}	7.10 ± 0.95^{a}	1.09 ± 0.15^{a}	9.54 ± 0.57^{a}
	0.90	2.43 ± 0.15^{a}	2.25 ± 0.14^{b}	2.26 ± 0.16^{b}	1.69 ± 0.41^{bc}	1.59 ± 0.14^{a}	1.98 ± 0.43^{b}
	0.94	5.07 ± 0.17^{b}	1.45 ± 0.18^{b}	5.68 ± 0.33^{c}	2.35 ± 0.10^{b}	6.03 ± 0.10^{b}	3.33 ± 0.25^{c}
	0.96	8.00 ± 1.38^{c}	1.49 ± 0.42^{b}	7.56 ± 0.52^{d}	1.07 ± 0.41^{cd}	8.08 ± 0.97^{c}	0.86 ± 0.32^{d}
	0.98	11.74 ± 0.52^{d}	1.35 ± 0.09^{b}	9.04 ± 0.37^{e}	0.50 ± 0.06^{d}	8.74 ± 0.51^{c}	0.71 ± 0.14^{d}
30	0.83	–	–	–	–	–	–
	0.86	1.25 ± 0.08^{a}	3.37 ± 0.34^{b}	1.18 ± 0.15^{a}	2.32 ± 0.44^{ab}	0.62 ± 0.05^{a}	1.68 ± 0.76^{b}
	0.90	4.68 ± 0.11^{b}	3.99 ± 0.34^{a}	3.17 ± 0.25^{a}	2.57 ± 0.46^{a}	2.33 ± 0.26^{b}	2.59 ± 0.49^{a}
	0.94	8.97 ± 0.16^{c}	1.43 ± 0.13^{c}	8.31 ± 0.22^{b}	1.45 ± 0.10^{bc}	7.46 ± 0.16^{c}	1.45 ± 0.09^{b}
	0.96	10.46 ± 1.20^{d}	1.27 ± 0.36^{c}	12.54 ± 2.65^{c}	1.54 ± 0.71^{bc}	11.97 ± 1.14^{d}	1.07 ± 0.27^{b}
	0.98	12.51 ± 0.63^{e}	0.42 ± 0.13^{d}	13.69 ± 0.66^{c}	0.85 ± 0.10^{c}	12.79 ± 0.18^{d}	0.87 ± 0.07^{b}
35	0.83	–	–	–	–	–	–
	0.86	1.07 ± 0.12^{a}	2.67 ± 0.44^{a}	0.66 ± 0.12^{a}	0.55 ± 0.47^{a}	0.67 ± 0.08^{a}	2.85 ± 0.79^{a}
	0.90	4.44 ± 0.14^{b}	1.99 ± 0.25^{b}	2.75 ± 0.05^{b}	0.99 ± 0.47^{ab}	2.22 ± 0.10^{b}	1.67 ± 0.19^{b}
	0.94	8.85 ± 0.14^{c}	1.19 ± 0.09^{c}	8.86 ± 0.04^{c}	1.32 ± 0.25^{b}	7.54 ± 0.46^{c}	1.29 ± 0.03^{bc}
	0.96	12.30 ± 1.53^{d}	1.10 ± 0.32^{cd}	16.98 ± 1.68^{d}	1.06 ± 0.04^{ab}	10.76 ± 0.75^{d}	0.11 ± 0.41^{bc}
	0.98	15.68 ± 1.53^{e}	0.54 ± 0.15^{d}	19.75 ± 1.35^{e}	1.04 ± 0.14^{ab}	15.42 ± 0.14^{e}	0.69 ± 0.04^{c}

No growth was observed for 28 days. SD: standard deviation.
For each strain and temperature level, different letters next to the means mean significant differences ($p<0.05$) among growth at the different a_w levels according to Tukey HSD test.

Table 3
Parameters estimated by the cardinal model applied to growth rates of *A. flavus* isolates (estimated parameter ± standard error).

Parameters	BAFC4273		BAFC4274		BAFC4275	
	CYA	CEM	CYA	CEM	CYA	CEM
μ_{opt}(mm/d)	19.96 ± 1.07	17.34 ± 6.43	19.69 ± 1.55	19.63 ± 1.78	18.27 ± 0.66	15.14 ± 1.31
T_{max} (°C)	40.20 ± 0.10	40.00 ± 0.10	40.40 ± 0.10	40.00 ± 0.00	40.40 ± 0.10	40.00 ± 0.10
T_{min} (°C)	8.40 ± 1.10	8.40 ± 1.40	10.03 ± 1.00	10.00 ± 1.70	9.40 ± 1.00	9.30 ± 1.30
T_{opt} (°C)	33.60 ± 0.30	33.50 ± 0.40	32.60 ± 0.30	35.50 ± 0.70	32.10 ± 0.30	33.20 ± 0.40
a_{wmin}	0.80 ± 0.01	0.81 ± 0.01	0.79 ± 0.01	0.85 ± 0.00	0.80 ± 0.01	0.85 ± 0.01
a_{wopt}	0.98 ± 0.00	1.00 ± 0.01	0.98 ± 0.01	0.98 ± 0.00	0.98 ± 0.01	0.98 ± 0.00
R^2	0.988	0.983	0.988	0.985	0.988	0.987
RMSE	0.750	0.703	0.724	0.782	0.717	0.600

much lower than the observed ones, as none of the isolates was able to grow at 10 °C. T_{max} was consistently predicted at 40 °C, which may be slightly low, although values over 40 °C were not tested in the study.

3.3. Modelling the growth/no-growth boundaries

Plots of probability of growth for temperature and a_w at 7, 14, 21 and 28 days of incubation for *A. flavus* BAFC4273 isolate is presented in Fig. 3. The other two isolates showed a similar response. It is graphically depicted that the probability plot shifted to lower a_w for the same temperature level as time advances, for all the assayed fungal isolates.

For a 4-week storage period, the probability of growth was always under 0.50 when water availability was under 0.81 a_w, although values under 0.79 a_w were required for a $p<0.10$ if no temperature control is exerted. As observed in the figures, probabilities of growth for the assayed isolates over 0.90 were predicted in the range 0.91–0.93 a_w at 23–33 °C in a week-period.

4. Discussion

Kinetic growth and growth/no growth models were described for *A. flavus* isolated from corn based feed products, in a synthetic (CYA) and a corn-based medium. Significant differences between the growth rates reached by the isolates in both media were observed, being higher in CYA than in CEM. CYA is a rich laboratory medium which probably encouraged *A. flavus* growth, whereas CEM might be closer to the nutrient levels in the real corn ecosystem. The development of predictive models in rich laboratory media may overestimate the ability of fungi to grow in foods, and lead to a predicted unrealistic broad range of growth conditions. García et al. (2011) validated a kinetic model for *A. parasiticus* and *A. ochraceus* developed on MEA in sterile maize grain, and they confirmed that growth of the fungi in maize was in general much slower than predicted by the models. The use of a food-analogue media, as CEM in the present study may be more convenient.

Maximum growth rate data were fitted to a cardinal model; the cardinal estimated parameters were sometimes not concordant with the observed ones, e.g. T_{min}, T_{max}, a_{wmin}, probably due to the limited number of experimental points. Estimated T_{min} values were in the range 8–10 °C, while T_{min} values for *A. flavus* have been previously reported at 12 °C on potato dextrose agar (Sautour et al., 2002) and at 13.2 °C on paddy (Mousa et al., 2011). It has been noted in some publications (Ratkowsky et al., 2005) that T_{min}, as well as the other cardinal parameters, is a notional, or theoretical, minimum temperature for bacterial growth and its estimate is typical several degrees below the minimum temperature at which growth is observed. The latter temperature can be described as MIN_t (Ross et al., 2011). Whereas T_{min} appears to be constant regardless of the effects on growth rate of other environmental factors, MIN_t is known to increase systematically as other environmental factors become more inhibitory to growth (Le Marc et al., 2002). That is the reason why the use of growth/no-growth models may be of more interest for application in food safety, as foods are usually stored under marginal conditions of either a_w or temperature. Regarding the other parameter which is determinant for the aims of the present study, a_{wmin}, its value was accurately estimated by the model, if we observe the experimental data. a_{wmin} values for *A. flavus* have been previously reported at 0.82–0.83 on potato dextrose agar (Sautour et al., 2001, 2002), and at 0.83–0.85 on paddy and chilli extract agar medium (Mousa et al., 2011; Marín et al., 2009). In order to compare the output of both cardinal and growth/no-growth models, the probabilities estimated by the second were calculated for the estimated a_{wmin}. While probabilities of growth as high as 0.99 were observed at a_{wmin} (0.85) for strains BAFC4274 and BAFC4275, an estimation of $a_{wmin} = 0.81$ for strain BAFC4273 corresponded to a probability of growth under this a_w level up to 0.17. Growth rates values in maize grain were only reported in Samapundo et al. (2007a,b); their observed values were comparable to those estimated in the present study, in the range 0.921–0.982 a_w and 22–30 °C. At 16 and 37 °C, however, the growth rates estimated in our study were higher than those in maize grain, while at 0.855–0.893 a_w, the results reported by Samapundo et al. (2007a,b) were, in general, higher. Regarding the probability model, it can be concluded that for safe storage of crops such as corn, a a_w <0.79 should be maintained but a_w as high

Table 4
Validation of the CEM probability model (at 28 days) on *A. flavus* growth data on corn from the literature. Existing studies showing growth (1) or no-growth (0)/estimated probability. Characters in bold highlight no concordance between observed and predicted values.

	16 °C	20 °C	22 °C	25 °C	26 °C	30 °C	32 °C	37 °C
0.69 a_w	0/0	–	–	–	0/0	–	0/0	–
0.73 a_w	0/0	–	–	–	0/0	–	0/0	–
0.75 a_w	0/0	–	–	–	1/0	–	1/0	–
0.77 a_w	0/0	–	–	–	1/0	–	1/0	–
0.80 a_w	1/0	–	–	–	1/0	–	1/0.11	–
0.85 a_w	1/0	–	–	1/0.79	1/0.88	–	1/0.97	–
0.86 a_w	0/0	–	1/0.43	1/0.93	–	1/0.99	–	1/0.83
0.88 a_w	–	1/0.51	–	–	–	1/1	–	–
0.89 a_w	0.5/0.01	–	1/0.98	1/1	1/1	1/1	1/1	–
0.90 a_w	–	–	–	1/1	–	–	–	–
0.92 a_w	1/0.33	–	1/1	1/1	–	1/1	–	1/0.98
0.93 a_w	–	–	–		–	1/1	–	–
0.94 a_w	–	–	–	1/1	–	–	–	1/0.97
0.95 a_w	1/0.95	–	1/1	1/1	–	1/1	–	–
0.96 a_w	–	–	–	1/1	–	–	–	–
0.97 a_w	–	–	–	1/1	–	1/1	–	1/0.95
0.98 a_w	1/1	1/1	1/1	1/1	–	1/1	–	–
0.99 a_w	–	–	–	1/1	–	1/1	–	–

Growth/no-growth values extracted from: Bluma and Etcheverry (2006, 2008), Giorni et al. (2008), Moretzsohn De Castro et al. (2002), Nesci et al. (2007, 2009), Oyebanji and Efiuvwevwere (1999), Samapundo et al. (2007a,b), Trucksess et al. (1988). Duration of the experiments ranged from 11 to 180 days.

as 0.83 could be permitted if temperatures over 22 °C are avoided; alternatively, cool storage (<10 °C) could be applied (values based on experiments on both CYA and CEM and p of growth <0.10). If only data obtained in CEM are considered, it allows predicting safe storage (p of growth <0.01) for one month of moist maize (e.g. 0.90 a_w) as long as temperature is under 15 °C, or of dry maize (e.g. 0.80 a_w) as long as temperature is under 27 °C. Storage at <0.77 a_w would be safe regardless of the storage temperature. Probability models allow evaluating the risk of fungal contamination in the process of storage, so the results obtained in this study may be useful for application in systems of food safety management. For evaluation of the performance of the probability model, the results were compared to existing studies in the literature reporting either growth or no-growth situations under different temperature/a_w levels in maize grain (Table 4). Despite the impact of aflatoxins in food safety, such studies carried out in maize grain were surprisingly scarce. Out of 56 conditions from which data in maize were available, 10 predicted values were not concordant with the observed ones (18%); 8 of those cases were linked to Trucksess et al. (1988) study, which has been pointed out before (Giorni et al., 2011) as reporting growth at unusually low a_w levels. Apart from those, the two non-concordant values were at 16 °C/0.92 a_w and at 22 °C/0.86 a_w, at the boundary of growth. Although the three *A. flavus* isolates tested in this study were not significantly different in terms of growth, which gives more relevance to the predictive models presented, it must be kept in mind that a higher number of isolates would be required to better represent the intraspecific variability and for a better prediction performance, including strains from different geographical origin if the models are to be applied at a worldwide level.

Acknowledgement

The authors are grateful to "Latinamerican Science and Technology Development Programme" (CYTED) and to "Consejo Nacional de Investigaciones Científicas y Técnicas" (CONICET) for the financial support. The authors are grateful to the European Union (MYCORED KBBE-2007-2-5-05 project), and Spanish government (AGL2010-22182-C04-04 project) for the financial support.

References

Astoreca, A., Dalcero, A., Fernández Pinto, V., Vaamonde, G., 2011. A survey on distribution and toxigenicity of *Aspergillus* section *Flavi* in poultry feeds. International Journal of Food Microbiology 146, 38–43.

Baranyi, J., Roberts, T.A., 1994. A dynamic approach to predicting bacterial growth in food. International Journal of Food Microbiology 23, 277–294.

Baranyi, J., Gibson, A.M., Pitt, J.I., Eyles, M.J., Roberts, T.A., 1997. Predictive models as means of measuring the relatedness of some *Aspergillus* species. Food Microbiology 14, 347–351.

Battey, A.S., Duffy, S., Schaffner, D.W., 2002. Modeling yeast spoilage in cold-filled ready-to drink beverages with *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, and *Candida lipolytica*. Applied and Environmental Microbiology 68, 1901–1906.

Bluma, R.V., Etcheverry, M.G., 2006. Influence of *Bacillus* spp. isolated from maize agroecosystem on growth and aflatoxin B_1 production by *Aspergillus* section *Flavi*. Pest Management Science 62, 242–251.

Bluma, R.V., Etcheverry, M.G., 2008. Application of essential oils in maize grain: impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. Food Microbiology 25, 324–334.

Dantigny, P., Guilmart, A., Radoi, F., Bensoussan, M., Zwietering, M., 2005. Modelling the effect of ethanol on growth rate of food spoilage moulds. International Journal of Food Microbiology 98, 261–269.

Donner, M., Atehnkeng, J., Sikora, R.A., Bandyopadhyay, R., Cotty, P.J., 2009. Distribution of *Aspergillus* section *Flavi* in soils of maize fields in three agroecological zones of Nigeria. Soil Biology and Biochemistry 41, 37–44.

Dorner, J.W., Cole, R.J., Lomax, L.G., Gosser, H.S., Diener, U.l., 1983. Cyclopiazonic acid production by *Aspergillus flavus* and its effects on broiler chickens. Applied and Environmental Microbiology 983, 698–703.

FAO, 2002. FAOSTAT Database. Food and Agricultural Organisation, Roma, Italy. URL: http://apps.fao.org/page/collections. 17 April 2003.

García, D., Ramos, A.J., Sanchís, V., Marín, S., 2009. Predicting mycotoxins in foods: a review. Food Microbiology 26, 757–769.

García, D., Ramos, A.J., Sanchís, V., Marín, S., 2011. Modelling the effect of temperature and water activity in the growth boundaries of *Aspergillus ochraceus* and *Aspergillus parasiticus*. Food Microbiology 28, 406–417.

Gibson, A.M., Baranyi, J., Pitt, J.I., Eyles, M.J., Roberts, T.A., 1994. Predicting fungal growth: the effects of water activity on *Aspergillus flavus* and related species. International Journal of Food Microbiology 23, 419–431.

Giorni, P., Battilani, P., Pietri, A., Magan, N., 2008. Effect of a_w and CO_2 level on *Aspergillus flavus* growth and aflatoxin production in high moisture maize post-harvest. International Journal of Food Microbiology 122, 109–113.

Giorni, P., Magan, N., Pietri, A., Battilani, P., 2011. Growth and aflatoxin production of an Italian strain of *Aspergillus flavus*: influence of ecological factors and nutritional substrates. World Mycotoxin Journal 4, 425–432.

Holmquist, G.U., Walker, H.W., Stahr, H.M., 1983. Influence of temperature, pH, water activity and antifungal agents on growth of *Aspergillus flavus* and *A. parasiticus*. Journal of Food Science 48, 778–782.

Jaime-Garcia, R., Cotty, P.J., 2010. Crop rotation and soil temperature influence the community structure of *Aspergillus flavus* in soil. Soil Biology and Biochemistry 42, 1842–1847.

JECFA, 1997. Evaluation of certain food additives and contaminants. Forty-sixth Report of the Joint FAO/WHO Expert Committee on Food Additives 1996. : WHO Technical Report Series, 868. World Health Organization, Geneva.

Lacey, J., Ramakrishna, N., Hamer, A., Magan, N., Marfleet, L.C., 1991. Grain fungi. In: Arora, D.K., Mukerji, K.G., Marth, E.H. (Eds.), Handbook of Applied Mycology: Foods and Feeds, vol. 3. Marcel Dekker, Inc., New York, pp. 121–178.

Le Marc, Y., Huchet, V., Bourgeois, C.M., Guyonnet, J.P., Mafart, P., Thuault, D., 2002. Modelling the growth kinetics of Listeria as a function of temperature, pH and organic acid concentration. International Journal of Food Microbiology 73, 219–237.

Marín, S., Sanchís, V., Teixido, A., Saenz, R., Ramos, A.J., Vinas, I., Magan, N., 1998. Colonisation of maize grain by *F. moniliforme* and *F. proliferatum* in the presence of competing fungi and their impact on fumonisin production. Journal of Food Protection 61, 1489–1496.

Marín, S., Colom, C., Sanchís, V., Ramos, A.J., 2009. Modelling of growth of aflatoxigenic *A. flavus* isolates from red chilli powder as a function of water availability. International Journal of Food Microbiology 128, 491–496.

Moretzsohn De Castro, M.F.P., Bragagnolo, N., De Toledo Valentini, S.R., 2002. The relationship between fungi growth and aflatoxin production with ergosterol content of corn grains. Brazilian Journal of Microbiology 33, 22–26.

Mousa, W., Ghazali, F.M., Jinap, S., Ghazali, H.M., Radu, S., 2011. Modelling the effect of water activity and temperature on growth rate and aflatoxin production by two isolates of *Aspergillus flavus* on paddy. Journal of Applied Microbiology 111, 1262–1274.

Nesci, A., Etcheverry, M., 2002. *Aspergillus* section *Flavi* populations from field maize in Argentina. Letters in Applied Microbiology 34, 343–348.

Nesci, A., Gsponer, N., Etcheverry, M., 2007. Natural maize phenolic acids for control of aflatoxigenic fungi on maize. Journal of Food Science 72, M180–M185.

Nesci, A., Marín, S., Etcheverry, M., Sanchís, V., 2009. Natural maize phytochemicals for control of maize mycoflora and aflatoxigenic fungi. World Mycotoxin Journal 2, 305–312.

Niles, E.V., Norman, J.A., Pimbley, D., 1985. Growth and aflatoxin production of *Aspergillus flavus* on wheat and barley. Transactions of the British Mycological Society 84, 259–266.

Oyebanji, A.O., Efiuvwevwere, B.J.O., 1999. Growth of spoilage mould and aflatoxin B_1 production in naturally contaminated or artificially inoculated maize as influenced by moisture content under ambient tropical condition. International Biodeterioration and Biodegradation 44, 209–217.

Panagou, E.Z., Skandamis, P.N., Nychas, G.J.E., 2003. Modelling the combined effect of temperature, pH and a_w on the growth rate of *Monascus ruber*, a heat-resistant fungus isolated from green table lives. Journal of Applied Microbiology 94, 146–156.

Pitt, J., Miscamble, B., 1995. Water relations of *Aspergillus flavus* and closely related species. Journal of Food Protection 58, 86–90.

Plaza, P., Usall, J., Teixido, N., Vinas, I., 2003. Effect of water activity and temperature on germination and growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. Journal of Applied Microbiology 94, 549–554.

Rao, L.B., Husain, A., 1985. Presence of cyclopiazonic acid in kodo millet (*Paspalum scrobiculatum*) causing 'kodua poisoning' in man and its production by associated fungi. Mycopathologia 89, 177–180.

Ratkowsky, D., Ross, T., 1995. Modelling the bacterial growth/no growth interface. Letters in Applied Microbiology 20, 29–33.

Ratkowsky, D.A., Olley, J., Ross, T., 2005. Unifying temperature effects on the growth rate of bacteria and the stability of globular proteins. Journal of Theoretical Biology 233, 351–362.

Ross, T., Olley, J., McMeekin, T.A., Ratkowsky, D.A., 2011. Some comments on Huang, L. (2010). Growth kinetics of *Escherichia coli* O157: H7 in mechanically-tenderized beef. International Journal of Food Microbiology 140, 40–48 International Journal of Food Microbiology 147, 78–80.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1995. Convenient model to describe the combined effects of temperature and pH on microbial growth. Applied and Environmental Microbiology 61, 610–616.

Samapundo, S., Devlieghere, F., Geeraerd, A.H., De Meulenaer, B., Van Impe, J.F., Debevere, J., 2007a. Modelling of the individual and combined effects of water activity and temperature on the radial growth of *Aspergillus flavus* and *A. parasiticus* on corn. Food Microbiology 24, 517–529.

Samapundo, S., Devlieghere, F., De Meulenaer, B., Debevere, J., 2007b. Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal. Food Microbiology 24, 336–345.

Sautour, M., Dantigny, P., Divies, C., Bensoussan, M., 2001. A temperature-type model for describing the relationship between fungal growth and water activity. International Journal of Food Microbiology 67, 63–69.

Sautour, M., Soares Mansur, C., Divies, C., Bensoussan, M., Dantigny, P., 2002. Comparison of the effects of temperature and water activity on growth rate of food spoilage moulds. Journal of Industrial Microbiology and Biotechnology 28, 311–316.

Trenk, H.L., Hartman, P.A., 1970. Effect of moisture content and temperature on aflatoxin production in corn. Applied Microbiology 19, 781–784.

Trucksess, M.W., Stoloff, L., Mislivec, P.B., 1988. Effect of temperature, water activity and other toxigenic mold species on growth of *Aspergillus flavus* and aflatoxin production on corn, pinto beans and soybeans. Journal of Food Protection 51, 361–363.

Yue, X., Sui, J., Niu, T., Liu, Y., Liu, X., 2011. Modeling the effect of temperature and water activity on the growth rate and lag phase of *Aspergillus flavus* during rice drying. Drying Technology 29, 1306–1312.

Zwietering, M.H., De Wit, J.C., Notermans, S., 1996. Application-of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption. International Journal of Food Microbiology 30, 55–70.