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Modelling of aflatoxin production by *Aspergillus parasiticus* in a solid medium at different temperatures, pH and propionic acid concentrations

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Abstract

The effect of pH (5.5 and 5.9), propionic acid concentration (129, 258 and 516 ppm) and temperature (25, 30 and 36 °C) on aflatoxin production by *Aspergillus parasiticus* in solid media at controlled water activity 0.95 was studied. Modelling of aflatoxin production was carried out using adequate equations to obtain the maximum aflatoxin concentration (C values) and the corresponding time to reach the maximum (*a* values). For control samples, the highest C values were observed for aflatoxin B1 and G1. The effect of temperature on aflatoxin production at different pH was analysed using a function derived from the Arrhenius equation. Optimum temperatures for aflatoxin production were 27.84 and 27.32 °C at pH 5.9 and 5.5, respectively. Two indices were defined in order to analyse the effect of undissociated propionic acid concentration (uac) on *C* and *a* values. Propionic acid showed the highest inhibition on *C* values for aflatoxin G1 and on *a* values for aflatoxin B1. Concentrations over 60 ppm uac did not show an increase in both indices indicating that there is no need to increase the concentration of propionic acid over this value. These plots may be useful for technological purposes and to identify conditions (pH, propionic acid and storage temperature) to inhibit aflatoxin production in foodstuffs.

Keywords: Aflatoxin production; Mathematical modelling; Propionic acid

1. Introduction

Aflatoxins are among the most toxic mycotoxins that contaminate a wide variety of food and feed commodities and usually cause significant economic loss to farmers and processors alike. Aflatoxins are carcinogenic, mutagenic, teratogenic and hepatotoxic compounds formed as secondary metabolites during the growth of *Aspergillus flavus* and *Aspergillus parasiticus*. Assuming sufficient nutrients are available (as is usually the case with foods), mycelia growth and aflatoxin production are controlled primarily by temperature and water activity (a_w). Additional factors such as heat treatment, modified-atmosphere packaging or the presence of preservatives, also contribute. Many antifungal compounds have been recommended for high-moisture grains to prevent mould growth. In particular, propionic acid has been suggested by a number of investigators (Christensen, 1973; Vandegraft Hesseltine, & Shotwell, 1975). Although propionic acid exhibits antimycotic activity, its use is limited to foods in which the pH is fairly acidic, since it has virtually no activity at neutral or near neutral pH values. The effect of propionic acid in combination with temperature on the growth of *A. parasiticus* has been previously studied (Al-Hili & Smith, 1992; Buchanan & Ayres, 1976; Molina & Giannuzzi, 1999). However, controversial reports concerning sublethal concentrations of propionic acid on aflatoxin production were found (Al-Hili & Smith, 1979, 1992; Ghosh & Häggblom 1985).

Mathematical modelling has been used to predict the extent of fungal growth and invasion in foodstuffs as a function of environmental conditions. However, mathematical modelling for toxin formation may be particularly

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difficult because the regulation of secondary metabolism is poorly understood (Le Bars, 1988). Moreover, the relationship between the rates of primary and secondary metabolism is not clear. An interesting review about a descriptive model for growth and aflatoxin formation affected by environmental conditions was presented by Pitt (1993). Aflatoxin production generally rises during the logarithmic and deceleration phases of microbial growth (Shih & 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 aflatoxin formation mechanisms are difficult and the formulation of mathematical models is therefore challenging.

The objectives of the present study were: (1) to evaluate the application of mathematical models to fit changes in aflatoxin production (aflatoxins B1, B2, G1 and G2); (2) to determine the simultaneous effect of temperature (25, 30 and 36 °C) and propionic acid concentration (129, 258 and 516 ppm) on aflatoxin production by *A. parasiticus* in solid media at two pH (5.5 and 5.9) and a single water activity ($a_w = 0.95$); and (3) to quantify the effect of undissociated propionic acid concentration on the representative parameters of aflatoxin production using appropriate indices to quantify these affects.

2. Material and methods

2.1. Preparation of spore inoculum

A. parasiticus NRRL 2999 was obtained from Cátedra de Microbiología de Alimentos, Facultad de Ciencias Exactas, UBA, Argentina. Fungi were maintained at 4 °C on potato dextrose agar (PDA) slants (Merck, Germany) and transferred weekly. The inoculum was prepared by growing the fungi on PDA slants for 7 days at 36 °C. Cultures were washed with 5 ml of 0.01% (w/w) sodium lauryl sulfate in 1% (w/w) glucose solution. Spores were loosened by gently scraping with a spatula. The number of spores, about 10^3 per ml, was assessed by a haemocytometer.

2.2. Culture media

The basal medium contained malt extract (1%), yeast extract (2%) and agar (2%). Media of $a_w = 0.95$ were prepared according to González, Resnik, and Vaamonde (1987), supplementing the necessary amount of glucose to reach the desired a_w. The a_w level was determined with a Novasina Thermoconstanter Humidat TH2/TH1 (Novasina, Zurich, Switzerland) which was calibrated against saturated salt solutions with known a_w. The pH of the medium was adjusted to 5.5 and 5.9 with NaOH. The media were autoclaved at 121 °C for 15 min in closed containers to maintain $a_w = 0.95$.

Temperature, pH and propionic acid concentrations were examined by means of a full factorial design. Combinations between temperature (25, 30 and 36 ± 0.1 °C), pH (5.5 and 5.9) and ammonium propionate/propionic acid/inert solid (Fungistop) (control, 250, 750 and 1500 ppm) were assayed ($3 \times 2 \times 4 = 24$ experiments). The maximum propionic acid concentration assayed (1500 ppm) is within the highest concentration used in foods. All experiments were performed in duplicate.

2.3. Cultivation

Cultures were grown on standard Petri dishes (90 mm diameter) containing approximately 15 ml of solid medium. For each combination, three plates of medium were inoculated with 10 μl of the spore suspension, dispensed from a micropipette. Inoculated plates were incubated in an upright position at 25, 30 and 36 °C inside plastic boxes containing dishes of saturated solutions of acid disodium phosphate ($a_w = 0.95$) to prevent changes in a_w. No variation in a_w was observed during the incubation time. At different times during storage, three plates of each condition were removed to analyse aflatoxin production.

2.4. Aflatoxin determination

Toxins were extracted from inoculated plates by mixing the content of three plates with 30 ml of chloroform (Merck No. 2445) in a high-speed blender. The extract was filtered through a Whatman No. 4 paper, and the final extracts were evaporated by rotatory evaporation followed by a gentle stream of nitrogen. Aflatoxin detection was performed by thin-layer chromatography on Silica-gel plates (0.25 mm, Merck No. 105553) at room temperature in a saturated chamber. A mixture of chloroform (Merck No. 2445)-acetone (Merck No. 14; 9:1) was used as developing solvent. Aflatoxin concentration was estimated by comparison to a standard solution prepared by dissolving 50 μg/ml standard aflatoxin (Sigmal Chemical, St. Louis, MO) in toluene (Merck No. 1783)-acetonitrile (Merck No. 800.015; 98:2). The level of aflatoxins B1, B2, G1 and G2 were determined with a spectrodensitometer CAMAG TLC Scanner 3 with CATS Software (Switzerland). Measurements were performed through fluorescence at 366 nm.

2.5. Determination of propionic acid concentration

Commercial propionic acid (Fungistop) was used as preservative. The product is a fungicide composed of propionic acid/ammonium propioniate (14.7/27.3%) prepared on a granular solid carrier. In order to know

the exact amount of propionic acid added, preservative levels were determined by high performance liquid chromatography (HPLC) and expressed as ppm of propionic acid. Twenty-five milliters of 0.009 N H_2SO_4 (mobile phase) were added to 7 g of agar media and extracted for 1 h with agitation in a shaker (Rolco S.R.L.). Samples were centrifuged at $700\times g$ for 5 min according to a modification of the method of Bevilacqua and Califano (1989). The supernatant was filtered once through filter paper and twice through a 0.45-μm membrane filter (Milipore Waters Associates SM N11306). Duplicate analyses were performed for all samples. An HPLC (Shimadzu) was operated with an AMINEX HPX-87-Biorad column under the following conditions: 0.009N H_2SO_4 as mobile phase, 0.7 ml/min flow, 580–600 psi, between 58 and 62 °C.

2.6. Statistical analysis

Statistical analysis was carried out with the aid of SYSTAT software (SYSTAT Inc, 1990, version 5.0). ANOVA provided the coefficients and the corresponding standard deviations. Systat software provides, for each data fit, the residual sum of square (RSS). Degrees of freedom corresponded to the number of datum points minus the number of model parameters. Percentage variance (%V) between both observed and predicted values is given by the following equation (Snedecor & Cochran, 1969):

$$\%V = 1 - \left[(1 - r^2)(n-1)/(n-N-1)\right] \times 100 \qquad (1)$$

where n = number of observations, N = number of terms, and r^2 = multiple regression coefficient.

3. Results and discussion

3.1. Modelling of aflatoxin production

Time course data for aflatoxin production usually exhibit a rise in concentration to a peak level followed by decay in concentration to near zero. Similar patterns were demonstrated for aflatoxins (Koehler et al. 1985; Park & Bullerman, 1983; Pitt, 1993) and for other mycotoxins such as citrinin (Damoglou et al., 1984),

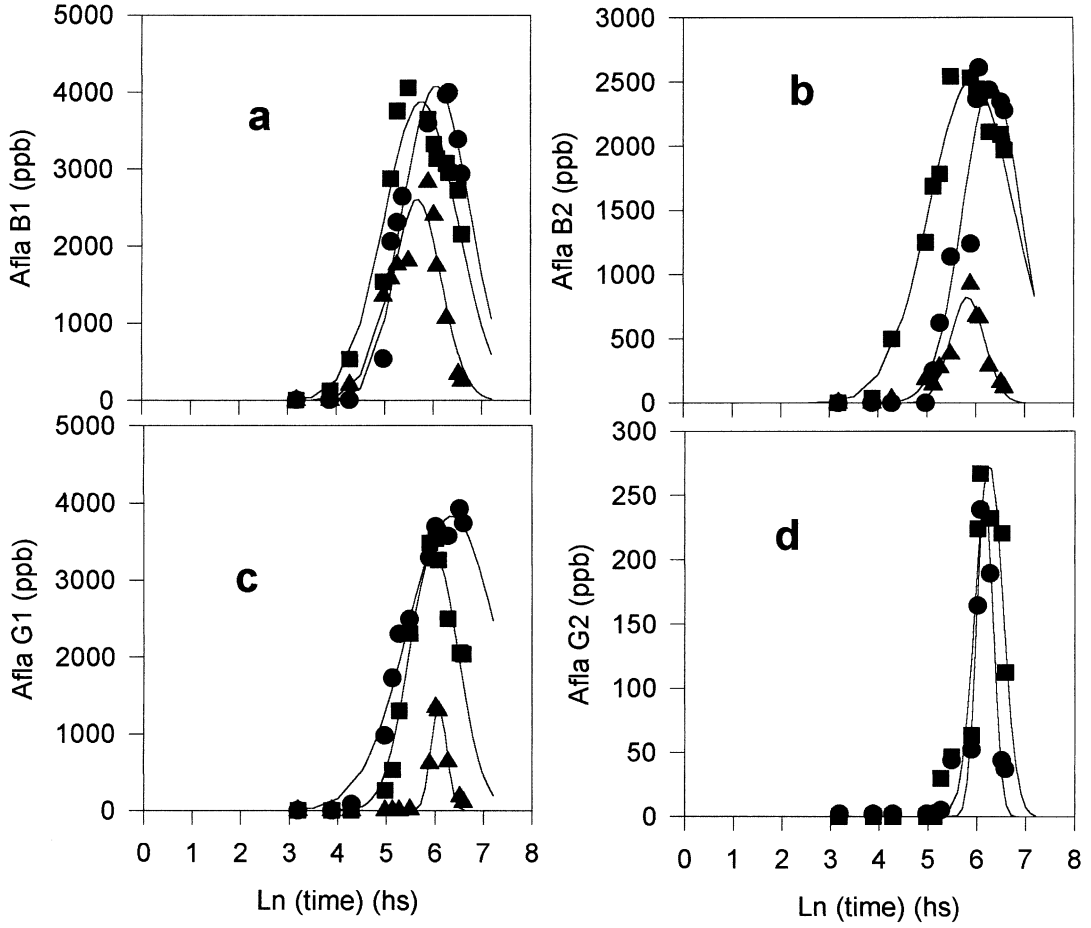

Fig. 1. Fitting of Eq. (2) to experimental data corresponding to aflatoxin production: (a) aflatoxin B1, (b) aflatoxin B2, (c) aflatoxin G1 and (d) aflatoxin G2, obtained in solid media at pH 5.9 at ■ 25, ● 30 and ▲ 36 °C.

deoxinivalenol (Greenhalgh et al., 1983), alternariol (Magan & Lacey, 1985, Chap. 23) and zearalenone (Montani et al., 1988a). Several models were used to study the production of fungal toxins. Logistc equations were used by Yousef and Marth (1983) and Montani et al. (1988b). The model represents a S-shaped curve excluding the phase of degradation. Pitt developed a model for aflatoxin production consisting of differential equations for the instantaneous change rates of mold mass and aflatoxin concentration as a function of the environmental conditions. However, equations from the mathematical model must be numerically solved by the aid of a computer. No simple equation for aflatoxin production was found in literature. In the present study, the different phases of synthesis and degradation of aflatoxin by *A. parasiticus* as a function of time was expressed by the following function.

$$\text{Aflatoxin concentration} = C \times \exp[-1\text{n}(t-a)/b] \quad (2)$$

where C (ppb) is the maximum aflatoxin concentration, a (hs) is the time to reach the maximum aflatoxin production (obtained by setting the derivative of Eq. (2) to zero), b is a constant and t is time. The adaptation of the proposed model to the experimental data for the aflatoxin (B1, B2, G1 and G2) production in agar media without addition of propionic acid (control) during storage at three temperatures (25, 30 and 36 °C) and at pH 5.9 and 5.5 are shown in Figs. 1 and 2, respectively. A good data fitting to the proposed model was observed. Both observed and calculated aflatoxin levels obtained from the model are shown in Fig. 3. Table 1 shows the values of C, a and b parameters and the corresponding statistical parameters obtained from Eq. (2). Such parameters allowed the calculation of the complete curve of aflatoxin production.

The model could be used in other experimental condition as well as for other toxins. Fig. 4 shows the application of the model to the 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., 1988b). R^2 values ranged between 0.989 and 0.999, and% variance for Eq. (1) was 98.7%, indicating a good correlation.

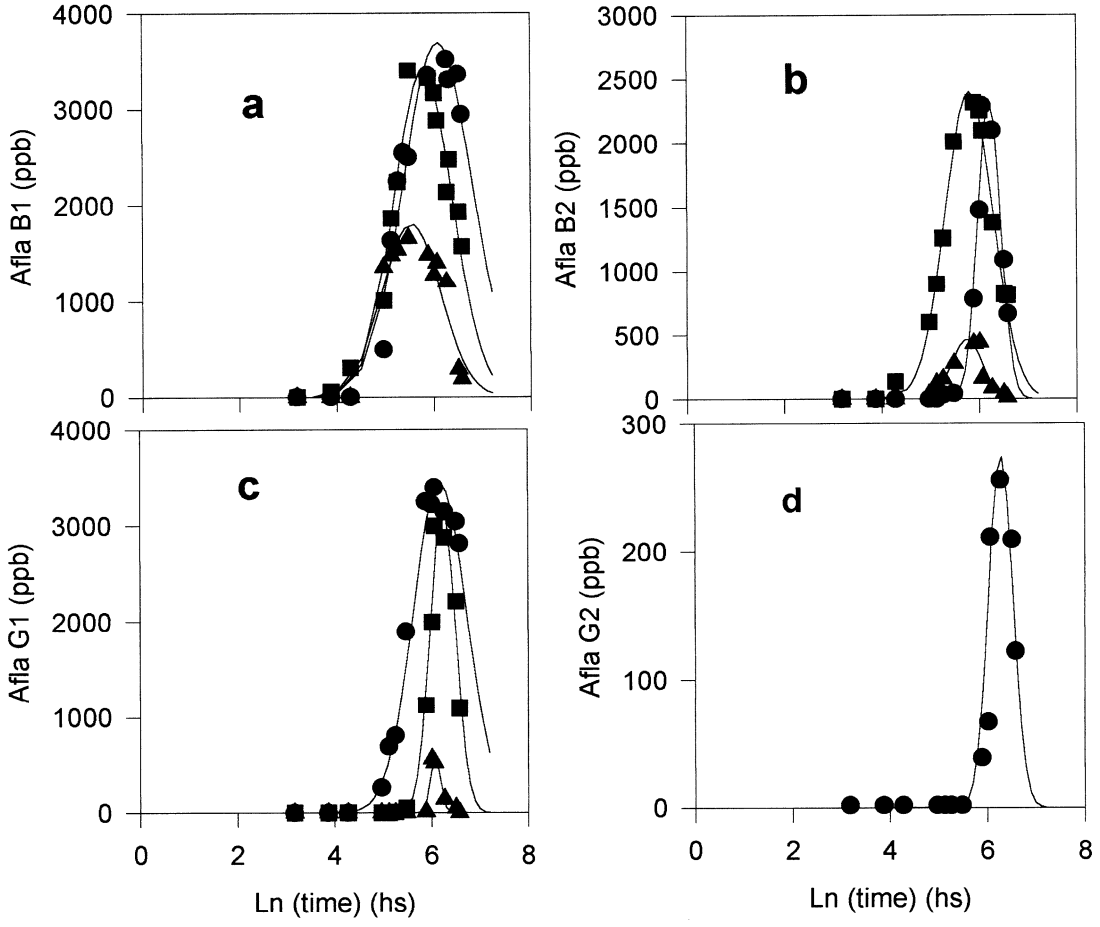

Fig. 2. Fitting of Eq. (2) to experimental data corresponding to aflatoxin production: (a) aflatoxin B1, (b) aflatoxin B2, (c) aflatoxin G1 and (d) aflatoxin G2, obtained in solid media at pH 5.5 at ■ 25, ● 30 and ▲ 36 °C.

3.2. Effect of temperature and pH on aflatoxin production

The effect of temperature on aflatoxin (B1, B2, G1 and G2) production in agar media at different pH (5.5 and 5.9) was studied. Maximum aflatoxin production (C values) was obtained between 25 and 30 °C for each aflatoxin and both pH; whereas lower values of maximum aflatoxin poduction were observed at 36 °C (Table 1). These results agree with those obtained by Faraj et al. (1991) for aflatoxin production (ppb) by cultures of *A. parasiticus* in irradiated maize seeds at $a_w = 0.95$ with levels of 4433, 3383 and 2367 ppb of aflatoxin at 25, 30 and 35 °C, respectively. Aflatoxin production was variable with optima ranging between 25 and 30 °C at $a_w = 0.95$ (Faraj et al., 1991).

In the present work a decrease in C and a parameters was observed for B1, B2, G1 and G2 aflatoxins with increasing temperature. No aflatoxin G2 production was observed at pH 5.9 and 36 °C nor at pH 5.5 and 30 or 36 °C (Figs. 1d and 2d).

For B1, B2, G1 and G2 a higher aflatoxin production (C values) was observed at pH 5.9. At pH of 5.5, values of C decreased at 25, 30 and 36 °C in comparison to pH 5.9. They were 9, 13, and 30%, for B1; 11, 7 and 57% for B2; and 11, 13 and 57% for G1, respectively. These results agreed with Buchanan and Ayres (1975), who reported that optimum pH for toxicogenesis was lower than 6.0, and a decrease in pH had a stronger effect on toxicogenesis.

For all conditions of pH and temperature, the highest aflatoxin production was observed for B1 and G1 (Table 1).

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).

$$f_T = a \times \exp\left\{-\left[\alpha^2/(T - T_{min}) + (\alpha^2/(T_{max} - T)\right]\right\} \quad (3)$$

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

Data obtained in the present work for B1, B2, G1 and G2 and data of Schindler et al. (1967) for relative aflatoxin formation and the function f_T, T_{min}, T_{max}, α and a fitted by nonlinear regression for pH 5.9 and 5.5 are show in Fig. 5a and b, respectively. Those data points

Table 1
Parameters obtained from the application of Eq. (2) to experimental data for aflatoxin production

Aflatoxin	Parameters[a]	pH 5.9			pH 5.5		
		25 °C	30 °C	36 °C	25 °C	30 °C	36 °C
B1	C	4064±205	3834±203	2613±233	3695±213	3339±142	1816±109
	a	6.06±0.04	5.75±0.05	5.66±0.04	6.09±0.06	5.79±0.03	5.54±0.04
	b	0.96±0.16	1.15±0.26	0.49±0.11	0.99±0.22	0.75±0.09	0.71±0.11
	d.f.	9	11	10	10	11	10
	R^2	0.989	0.985	0.96	0.984	0.991	0.979
	%V	98	98	94	98	99	99
B2	C	2624±135	2578±74	1040±116	2348±117	2405±54	445±55
	a	6.20±0.03	5.80±0.03	5.66±0.02	6.21±0.01	5.80±0.01	5.75±0.03
	b	0.61±0.18	1.42±0.09	0.16±0.03	0.11±0.01	0.47±0.03	0.19±0.05
	d.f.	10	10	10	10	10	10
	R^2	0.978	0.988	0.966	0.987	0.997	0.936
	%V	99	99	97	99	99	95
G1	C	3867±81	3335±130	1357±55	3443±68	2897±198	588±48
	a	6.15±0.08	6.01±0.03	6.07±0.09	6.18±0.02	6.15±0.05	6.08±0.01
	b	1.22±0.25	0.53±0.06	0.05±0.05	0.70±0.06	0.12±0.09	0.02±0.01
	d.f.	10	10	10	10	10	10
	R^2	0.996	0.998	0.957	0.996	0.954	0.957
	%V	99	99	98	97	97	97
G2	C	620±21	251±39	ND[b]	401±81	ND	ND
	a	6.11±0.02	6.15±0.03	ND	6.13±0.08	ND	ND
	b	0.002±0.01	0.05±0.09	ND	0.01±0.001	ND	ND
	d.f.	9	10	–	10	–	–
	R^2	0.989	0.946	–	0.978	–	–
	%V	97	95	–	99	–	–

[a] d.f., degrees of freedom, $P < 0.001$; R^2 coefficient regression.
[b] ND, not detected.

corresponding to 25, 25 and 36 °C were obtained in the present work, whereas those corresponding to 13 and 18 °C were reproduced from Schindler et al. (1967). Table 2 shows the estimated and statistical parameters. The shape of the temperature function is controlled by α, and the optimum temperature falls halfway between T_{min} and T_{max}. Eq. (3) is symmetric about T_{opt}. The optimum temperature for toxicogenesis [obtained by setting the derivative of Eq. (3) to zero] is given by:

$$T_{opt} = (T_{max} + T_{min})/2 \quad (4)$$

T_{opt} values obtained through application of Eq. (4) were 27.84 and 27.32 °C at pH 5.9 and 5.5, respectively. It has been generally accepted that T_{opt} for aflatoxin production ranges between 25 and 28 °C (Gourama & Bullerman, 1995).

3.3. Determination of propionic acid concentration

Propionic acid is added to foodstuffs mixed with specific carriers in an inert solid. These carriers gradually liberate propionic acid gas enhancing its inhibitory power. The main advantage is that the vapors are not as corrosive as liquid propionic acid. In the present work, different concentrations of commercial propionic acid, i.e. 250, 750 and 1500 ppm, were added to the media and the corresponding levels of propionic acid measured by HPLC were 129, 258 and 516 ppm in agar media, respectively. It is well know that the antimicrobial effect of organic acids is mainly caused by the action of the undissociated fraction of the acid rather than by hydrogen ions. This is due to the increment of the undissociated portion of the weak acid, which has a higher ability to penetrate the cells than dissociation products. The undissociated concentration (uac) of a weak acid such as propionic acid (monoprotic), can be calculated through the following expression:

$$[AH] = Ca[H^+]/(Ka + [H^+]) \quad (5)$$

where [AH] is the undissociated concentration (ppm) and Ka is the equilibrium constant of propionic acid (pK = 4.75). Ca is the total acid concentration (ppm). Total acid concentration values obtained in the present work were: 129, 258 and 516 ppm. By application of Eq. (5) at both pH (5.5 and 5.9), the corresponding [AH] were: 11.2, 22.4, 24.9, 44.9, 49.5 and 99.8 ppm.

Table 2
Parameters obtained for the effect of temperature on aflatoxin production by application of Eq. (3)

Parameters	pH 5.9	pH 5.5
A	8.04	13.09
α	3.91	4.29
T_{min}	12.99	12.75
T_{max}	42.67	41.90
R^2	0.986	0.980
$\%V$	97	95

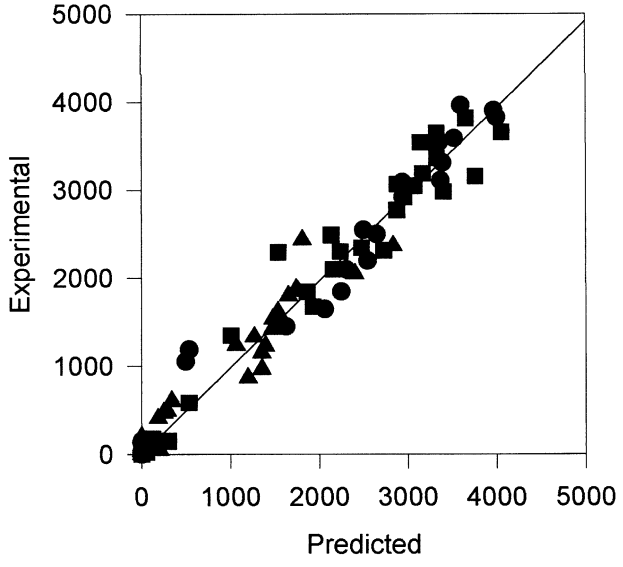

Fig. 3. Correlation between experimental data and predicted values corresponding to aflatoxin B1, B2, G1 and G2 production. Estimated data were obtained through application of Eq. (2) for control samples at ■ 25, ● 30 and ▲ 36 °C.

3.4. Inhibitory effect of propionic acid

Eq. (2) was fitted to aflatoxin production (B1, B2, G1 and G2) which were collected at different undissociated acid concentration of propionic acid at 25, 30 and 36 °C. For all aflatoxins, experimental data and predicted values showed a good correlation. A minimum of 12 data points was employed to fit the equation. Table 3

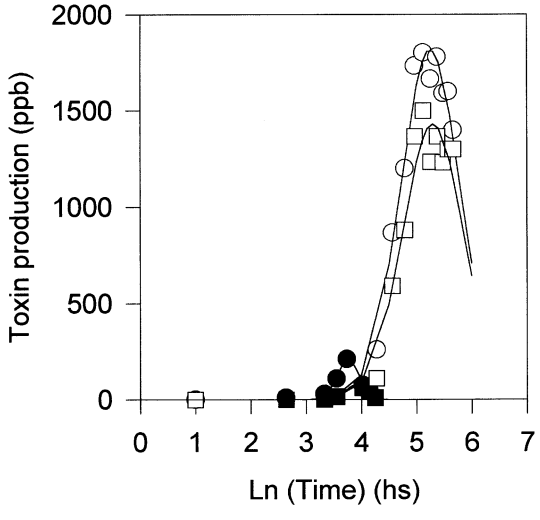

Fig. 4. Fitting of Eq. (2) to experimental data corresponding to aflatoxin production obtained by Faraj et al. (1991) ○ 25 °C, □ 35 °C and experimental data for Zearalenone production obtained by Montani et. al. (1988a) at ● 15 and ■ 25 °C.

shows the parameters obtained from the model (C, b and a values). An increase of undissociated acid concentration produced a diminution in the maximum aflatoxin production (C values) and increased the time to reach the maximum levels (a values). It is generally accepted that propionic acid inhibits aflatoxin production (Zaika & Buchanan, 1987), even at sublethal concentrations (Ghosh & Häggblom, 1985); however, other researchers found that aflatoxin concentration was markedly increased in cultures of *A. flavus* incubated with suboptimal levels of propionic acid in liquid media (Al-Hilli & Smith, 1992). In the present work, the inhibitory effect of propionic acid on toxicogenesis was confirmed for *A. parasiticus* in solid media. The reason for the aforementioned discrepancy could be due to differences in culture conditions, the use of different strains, and a variation in the acid response among the fungal isolates.

3.5. Inhibition indices

In order to compare the effect of undissociated propionic acid concentration with respect to control samples for the different aflatoxins, the inhibitory index on parameter C (obtained applied Eq. (2)) $(II)_c$ was defined:

$$(II)c = [(Co - C)/Co] \times 100 \quad (6)$$

where Co correspond to C values obtained from Eq. (2) for the control sample (Table 1), and C correspond to values at different undissociated acid concentrations (Table 3). When $Co = C$, (II)c is zero and no inhibitory effect is observed. (II)c is 100 when C is near zero, and the effect of propionic acid is maximum. (II)c represents the

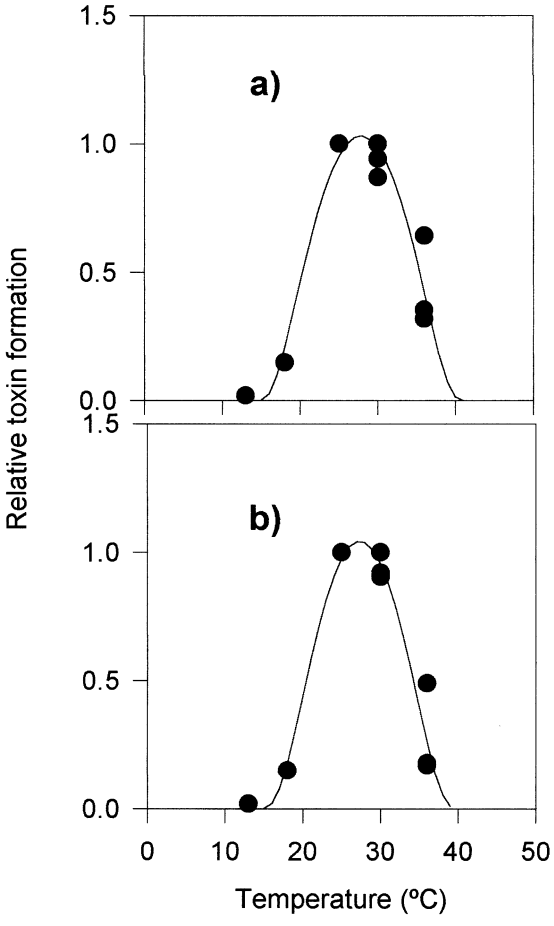

Fig. 5. Model functions for the effects of temperature on aflatoxin production. Data to 13 and 18 °C obtained from Schindler et al. (1967) and to 25, 30 and 36 °C from present work.

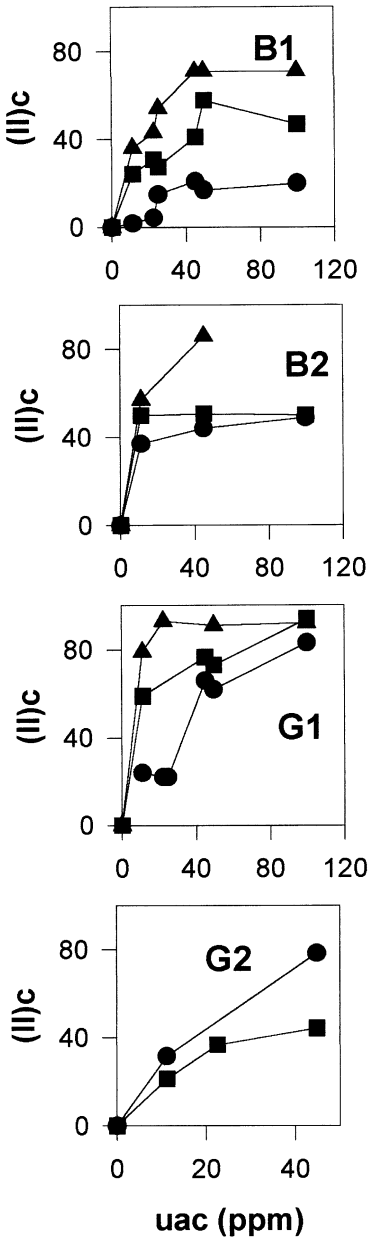

Fig. 6. Inhibition Index (IIc) for aflatoxin B1, B2, G1 and G2 as a function of undissociated acid concentration (uac) at ● 25 °C, ■ 30 °C, and ▲ 36 °C.

effect of propionic acid on the maximum aflatoxin production, but does not reflect the effect on the time to reach the maximum aflatoxin production (a values). Consequently, the inhibitory effect of a values could be defined:

$$(\mathrm{II})a = [(e^{a} - e^{ao})/e^{ao}] \times 100 \quad (7)$$

where a is the time necessary to reach the maximum production of aflatoxin in samples treated with different concentrations of undissociated propionic acid (Table 3) and ao the corresponding value for the control samples (Table 1). (II)a is zero when $a = ao$, indicating that both control and sample reach the maximum production at the same time. If this is the case, no effect of propionic acid on parameter a would be observed. When (II)a is positive, the maximum production of aflatoxin takes place at longer times than the control. (II)a has negative values when $a < ao$, and the time required to reach the maximum production is shorter that the corresponding to controls. Figs. 6 and 7 show the effect of undissociated acid concentration on both inhibition indices. The inhibition of both indices increased with temperature, except for G2. Aflatoxin G1 showed the strongest inhibition, and showed high values of (II)c at low acid concentrations; whereas B1 showed higher inhibition in (II)a. The effect of propionic acid on B1 and G1 has relevant toxicological implications because unsaturated compounds (B1 and G1) are approximately 4.5 times more effective than their respective dehydroderivatives (B2 and G2) according to Wogan (1966). No significant

Table 3
Parameters obtained by application of Eq. (2) to experimental data for aflatoxin production at different undissociated propionic acid concentration at 25, 30 and 36 °C

Aflatoxin	T (°C)	Parameters	Undissociated acid concentration of propionic acid (uac, ppm)					
			11.2	22.4	24.9	44.9	49.5	99.8
B1	25	C	3983±120	3881±171	3141±241	3213±106	3080±137	2952±198
		a	6.13±0.03	6.13±0.03	6.17±0.10	6.28±0.10	6.40±0.16	6.40±0.22
		b	0.78±0.01	0.57±0.07	0.96±0.03	1.75±0.38	1.35±0.42	1.22±0.56
	30	C	2904±157	2654±124	2424±87	2265±145	1413±147	1778±182
		a	5.90±0.04	5.96±0.31	6.19±0.06	6.25±0.05	6.22±0.05	6.24±0.02
		b	0.89±0.16	0.65±0.08	1.87±0.44	0.95±0.10	0.59±0.13	0.08±0.02
	36	C	1671±85	1489±144	835±65	760±124	ND[a]	ND
		a	5.66±0.31	5.85±0.03	5.78±0.02	5.99±0.01	ND	ND
		b	0.52±0.06	0.42±0.07	0.31±0.03	0.19±0.07	ND	ND
B2	25	C	1652±48	1532±67	1512±54	1480±140	1440±43	1457±25
		a	6.25±0.02	6.25±0.02	6.24±0.02	6.24±0.01	6.30±0.02	6.34±0.03
		b	0.09±0.01	0.23±0.02	0.11±0.01	0.10±0.01	0.12±0.06	0.21±0.06
	30	C	1267±133	1260±44	1250±72	1244±71	839±77	560±45
		a	5.83±0.03	5.85±0.03	5.85±0.02	5.86±0.02	5.85±0.02	5.85±0.02
		b	0.15±0.03	0.21±0.02	0.23±0.01	0.31±0.04	0.21±0.01	0.06±0.01
	36	C	446±44	350±32	340±37	155±14	ND	ND
		a	5.80±0.03	5.83±0.01	5.85±0.02	5.86±0.02	ND	ND
		b	0.15±0.03	0.12±0.02	0.15±9.01	0.12±0.01	ND	ND
G1	25	C	2935±125	3016±64	2701±192	1299±85	1303±92	578±172
		a	6.20±0.04	6.24±0.02	6.26±0.01	6.27±0.07	6.32±0.03	6.33±0.01
		b	0.56±0.09	0.49±0.03	0.13±0.02	1.17±0.21	0.19±0.04	0.07±0.03
	30	C	1370±46	1100±112	1010±98	768±154	791±103	171±142
		a	6.18±0.04	6.18±0.02	6.17±0.12	6.17±0.03	6.16±0.03	6.17±0.04
		b	0.69±0.09	0.45±0.09	010±0.05	0.04±0.02	0.09±0.03	0.29±0.08
	36	C	276±29	95±11	81±10	70±15	51±6	49±4
		a	6.12±0.02	6.12±0.03	6.12±0.03	6.12±0.02	6.12±0.02	6.12±0.05
		b	0.07±0.02	0.11±0.03	0.05±0.03	0.05±0.05	0.02±0.09	0.02±0.05
G2	25	C	425±39	352±43	328±34	210±32	134±10	ND
		a	6.23±0.01	6.25±0.02	6.29±0.02	6.30±0.02	6.31±0.02	ND
		b	0.14±0.14	0.10±0.09	0.11±0.02	0.05±0.04	0.02±0.05	ND
	30	C	207±27	159±21	140±10	ND	ND	ND
		a	6.21±0.02	6.30±0.03	6.38±0.06	ND	ND	ND
		b	0.12±0.28	0.13±0.04	0.39±0.13	ND	ND	ND
	36	C	ND	ND	ND	ND	ND	ND
		a	ND	ND	ND	ND	ND	ND
		b	ND	ND	ND	ND	ND	ND

[a] ND, not detected.

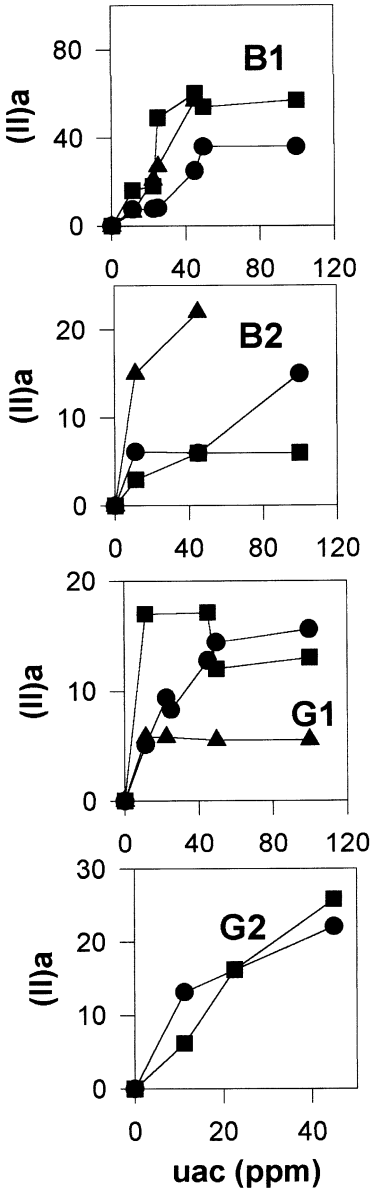

Fig. 7. Inhibition Index (IIa) for aflatoxin B1, B2, G1 and G2 as a function of undissociated acid concentration (uac) at ● 25 °C, ■ 30 °C, and ▲ 36 °C.

increment in both indexes was observed at concentrations over 60 ppm uac, suggesting that inhibition could be achieved at this concentration. These plots (Figs. 6 and 7) are useful for technological purposes and permit identification of conditions (propionic acid and temperature storage) that produce inhibition of aflatoxin production. This model has a practical application but future research efforts should be develope to validate the proposed models. Although the biochemical pathways of aflatoxin synthesis are now well documented, the physiological reasons for the synthesis are still unclear. Undoubtedly, growth conditions play a major role in the ability of fungi to produce secondary metabolites. The model proposed in the present work would allow the prediction of aflatoxin production under different conditions within the studied range of temperatures and propionic acid concentration.

4. Conclusion

Aflatoxin production was modelled using a non-linear model to fit aflatoxin accumulation in a solid media. Besides, parameters such maximum aflatoxin production and the time to reach these values were obtained. For control samples, the highest aflatoxin production was observed for B1 and G1.

The effect of temperature on aflatoxin production at different pH was analysed using an Arrhenius-derived equation. Optimum temperatures were 27.84 and 27.32 °C at pH 5.9 and 5.5, respectively.

The effect of undissociated propionic acid concentration was studied on the parameters obtained from the non-linear model. Two indices were defined in order to analyse the effect of undissociated propionic acid concentration. Production of Aflatoxin G1 was the most effectively inhibited; whereas aflatoxin B1 was more effectively inhibited on the time to reach the maximum aflatoxin production.

Concentrations over 60 ppm uac did not produce a significant increase in both indices, suggesting that it is unnecessary to use higher concentrations to inhibit aflatoxin production.

Indices plots are useful for technological purposes and permit treatment conditions (pH, propionic acid and temperature storage) that produce inhibition of aflatoxins production to be established.

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