ELSEVIER

International Journal of Food Microbiology 67 (2001) 63–69

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.nl/locate/ijfoodmicro

A temperature-type model for describing the relationship between fungal growth and water activity

Marc Sautour, Philippe Dantigny *, Charles Divies, Maurice Bensoussan

Laboratoire de Microbiologie, ENS.BANA UMR INRA 1082—Université de Bourgogne, 1 Esplanade Erasme, F-21000 Dijon, France

Received 20 February 2000; received in revised form 20 November 2000; accepted 15 December 2000

Abstract

Growth of *Penicillium chrysogenum*, *Aspergillus flavus*, *Cladosporium cladosporioides* and *Alternaria alternata* at their respective optimum temperatures was studied in Potato Dextrose Agar (PDA) medium at different water activities (a_w) adjusted with glycerol. The growth rate (μ) was expressed as the increase in colony radius per unit of time. This paper extends the model that showed the relationship between temperature and bacterial growth rate developed by Rosso et al. [J. Theor. Biol. 162 (1993) 447] to describe the influence of a_w on fungal development. An excellent correlation between the experimental data and the model predictions was obtained, the regression coefficients (r^2) were greater than 0.990, with the exception of that for *A. flavus* ($r^2 = 0.982$). In addition, the use of such a model allows predictions of the cardinal water activities: a_{wmin}, a_{wopt} and a_{wmax}. The estimation of the minimum water activity (a_{wmin}) was in accordance with data literature for all the moulds considered here, but seemed to be slightly underestimated for *P. chrysogenum* and *A. flavus* when compared to our experimental values. The estimations of the optimal water activity (a_{wopt}) and the optimal growth rate (μ_{opt}) were in excellent agreement to the experimental results for the four moulds. Through this example, it is suggested that the same approach for modelling can be used for various microorganisms (e.g. bacteria and moulds), and different environmental parameters (e.g. temperature and water activity).

Keywords: Fungal growth; Water activity; Predictive modelling

1. Introduction

For 15 years, predictive food microbiology has included the development of models capable of describing the growth of pathogenic bacteria (Buchanan and Phillips, 1990). However, predictive modelling of filamentous fungal growth has not received the same attention (Gibson et al., 1994; Gibson and Hocking, 1997). Two of the most important environmental parameters that determine the ability of moulds to grow on foods are water activity (a_w) and temperature (T) (Scott, 1957). An empirical approach to modelling the effects of a_w on mould growth was used by Gibson et al. (1994) who found that the logarithm of the fungal growth rate (μ, measured as the increase in colony diameter per unit of time) showed a parabolic relationship with the square root of

* Corresponding author. Tel: +33-380-396-679; fax: +33-380-396-640.
E-mail address: phdant@u-bourgogne.fr (P. Dantigny).

0168-1605/01/$ - see front matter
PII: S0168-1605(01)00471-8

$b_w = 1 - a_w$, leading to $\ln\mu = b_0 + b_1\sqrt{(1-a_w)} + b_2(1-a_w)$. They investigated the appropriateness of models that were previously used to predict bacterial growth for the interpretation of mould growth data.

Much work has been directed towards the development of models for bacterial growth as a function of temperature and a_w (McMeekin et al., 1987; Zwietering et al., 1994; Rosso et al., 1995). The temperature dependence of the specific growth rate of bacteria may be modeled by means of the square root model (Ratkowski et al., 1983) with the cardinal parameters (e.g. the minimum temperature for growth, T_{min} and the maximum temperature for growth, T_{max}) as follows: $\sqrt{\mu} = b(T - T_{min})[1 - \exp(c(T - T_{max}))]$, where b and c are design parameters. Rosso et al. (1993) proposed another model of temperature dependence that uses the three cardinal temperature (T_{min}, T_{max}, T_{opt}) and the specific growth rate at the optimum temperature (μ_{opt}) as parameters:

$$\mu = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\right]}. \tag{1}$$

Although this model is different from the Ratkowsky model, it usually fits the data equally well and has the advantage that the four parameters have a physiological meaning (Cuppers et al., 1997). In addition, the Rosso model provides an estimation of the optimal parameters (μ_{opt} and T_{opt}).

Cuppers et al. (1997) successfully combined the Rosso and Ratkowsky models to describe the combined effects of temperature and NaCl on the growth rate of some food spoilage moulds. However, our work only studies the effect of a_w on fungal growth. The four moulds used, *Penicillium chrysogenum*, *Cladosporium cladosporioides*, *Aspergillus flavus* and *Alternaria alternata*, were incubated at their optimum temperature for growth and at pH close to the optimum. The main objective of this paper was to determine whether the Rosso model could be extended to describe the relationship between growth rate and water activity and to estimate the cardinal water activities (a_{wmin}, a_{wmax}, a_{wopt}) for the moulds studied.

2. Materials and methods

2.1. Fungal isolates

P. chrysogenum, *A. flavus*, *C. cladosporioides* and *Alt. alternata* were isolated from spoiled pastry products and identified according to Samson et al. (1995).

2.2. Media and preparation

The moulds were maintained on Potato Dextrose Agar (PDA; BioMérieux, Marcy l'Etoile, France) at room temperature, from 17°C to 25°C. Experiments were carried out on Petri dishes containing PDA at the optimum temperatures: 25 ± 1°C for *P. chrysogenum* and *Alt. alternata*, 26 ± 1°C for *C. cladosporioides* and 31 ± 1°C for *A. flavus*. Media were adjusted to various a_w (e.g. 0.99, 0.985, 0.98 and then in decrements of 0.01 to a minimum of 0.83) by substituting a part of water by glycerol (w/w). Water activity measurements of the adjusted media were determined using an Aqualab CX2T (Decagon Devices, Pullman, WA, USA) with an accuracy of ±0.003.

To maintain the a_w of the culture medium at an exact value for the duration of the experiment, Petri dishes corresponding to a particular a_w value were placed in 1.5-l closed boxes in which relative humidity was controlled by a large volume of a glycerol–water solution. All experiments were performed in triplicate, for a maximum period of 3 weeks. The initial pH for all the experiments (5.8 ± 0.1) was close to the value of 5 considered as an optimum for most moulds (Lacey, 1989).

2.3. Assessment of hyphal growth rate

Growth was evaluated daily by visual measurement of the average increase of the fungal colony along two perpendicular diameters (Trinci, 1969; Gervais et al., 1988). Radius vs. time was plotted and radial growth rates (μ, mm day^{-1}) were evaluated from the slopes by linear regression.

2.4. Determination of optimum temperatures

The moulds were incubated on PDA at 0.99 a_w at 11 or more temperatures ranging from −2°C to 35°C for *P. chrysogenum*, *C. cladosporioides* and *Alt. alternata*, from 3°C to 40°C for *A. flavus*. Growth rates were plotted and the optimum temperatures were established.

2.5. Model

The relationship between the growth rate and water activity has been assessed using the following Rosso-type relationship:

$$\mu = \frac{\mu_{opt}(a_w - a_{wmax})(a_w - a_{wmin})^2}{(a_{wopt} - a_{wmin})[(a_{wopt} - a_{wmin})(a_w - a_{wopt}) - (a_{wopt} - a_{wmax})(a_{wopt} + a_{wmin} - 2a_w)]}. \quad (2)$$

2.6. Determination of optimum temperatures

To stabilise the variance of the growth rate, a logarithmic transformation was used as suggested by Zwietering et al. (1991) and Alber and Schaffner (1992).

$$\left[\ln\mu = \ln\frac{\mu_{opt}(a_w - a_{wmax})(a_w - a_{wmin})^2}{(a_{wopt} - a_{wmin})[(a_{wopt} - a_{wmin})(a_w - a_{wopt}) - (a_{wopt} - a_{wmax})(a_{wopt} + a_{wmin} - 2a_w)]}\right]. \quad (3)$$

The cardinal water activities (a_{wmin}, a_{wmax} and a_{wopt}) and the specific growth rates at the optimum temperatures (μ_{opt}) were estimated by means of nonlinear regression software based upon the Levenberg–Marquardt algorithm (SlideWrite 5.0, Advanced Graphics Software, Carlsbad, CA, USA) as described previously (Dantigny, 1998).

3. Results and discussion

3.1. Optimum growth temperatures

The optimum temperatures were found to be 25°C for *P. chrysogenum* and *Alt. alternata*, 26°C for *C. cladosporioides* and 31°C for *A. flavus*.

3.2. Growth rates

The results for growth rates of the four moulds are reported in Table 1. The 95% confidence intervals are all narrow, suggesting the suitability of the model. At 25°C, the growth rate for *A. flavus* was found to be equal to 4.37 mm day^{-1} (data not

Table 1
Estimation of the cardinal water activities and the optimum growth rate and 95% confidence intervals obtained for four moulds using the Rosso-type model (in Eq. (2))

Fungus	μ_{opt} (mm day^{-1})	a_{wmin}	a_{wopt}	a_{wmax}
P. chrysogenum	4.55 ± 0.15	0.810 ± 0.006	0.985 ± 0.002	0.991 ± 0.001
A. flavus	8.70 ± 0.46	0.822 ± 0.020	0.974 ± 0.004	0.993 ± 0.003
C. cladosporioides	4.46 ± 0.17	0.850 ± 0.007	0.983 ± 0.002	0.991 ± 0.001
Alt. alternata	4.62 ± 0.38	0.883 ± 0.005	0.987 ± 0.003	0.990 ± 0.002

shown). It appears that at 25–26°C, the radial growth rate of *A. flavus* and the optimal growth of the three other moulds, *P. chrysogenum*, *C. cladosporioides* and *Alt. alternata* are similar (4.37, 4.55, 4.46 and 4.62 mm day^{-1}, respectively). At 31°C, μ_{opt} obtained for *A. flavus* is about twice the value of μ_{opt} obtained for *P. chrysogenum*, *C. cladosporioides* and *Alt. alternata* at 26°C.

3.3. Cardinal water activities

The cardinal a_w values are reported in Table 1. For the moulds considered here, the maximum a_w can be considered as constant. *A. flavus* exhibits 95% confidence intervals for a_{wmin} and a_{wopt} as about twice the intervals found for *P. chrysogenum*, *C. cladosporioides* and *Alt. alternata*. Nevertheless, *A. flavus* is characterised by significantly lower a_{wopt} than the other three fungi.

Depending on the temperature and the nature of the a_w depressant, Gibson et al. (1994) reported optimum water activities (a_{wopt}) ranging from 0.980 to 0.995 for *A. flavus* and closely related species. Due to shortage of literature data, the estimated values have been compared to the experimental results shown in Fig. 1. The optimal water activity can be defined as the water activity at which the growth rate, μ_{opt}, is the greatest.

For *P. chrysogenum*, the average growth rates and standard deviations are 4.44 ± 0.05 and 4.54 ± 0.03 mm day^{-1} at a_w 0.98 and 0.985, respectively. Therefore, the experimental values are within the 95% confidence intervals reported in Table 1 for μ_{opt} and a_{wopt}.

For *A. flavus*, the definition as above mentioned for the optimum water activity leads to an experimental value of a_{wopt} equal to 0.98 for which the growth rate (defined as μ_{opt}) equals 8.58 ± 0.06 mm day^{-1}. The experimental value a_{wopt} of 0.98 is outside the 95% confidence interval, but no experiment was carried out at a_w 0.975. Should this experiment be carried out, the growth rate would likely have been greater than 8.08 mm day^{-1}, which was the average growth rate obtained at a_w 0.97, but this should be verified. In addition, the estimation of μ_{opt} for *A. flavus* is consistent with the experimental value.

For *C. cladosporioides*, the average growth rates were 4.42 ± 0.08 and 4.40 ± 0.16 mm day^{-1} at a_w 0.98 and 0.985, respectively. All the experimental values are within the 95% confidence intervals shown on Table 1.

Alt. alternata is characterised by a sharp decrease in the radial growth rate from a_{wopt} to a_{wmax}, as shown in Fig. 1. The model does not predict any significant difference between the estimations of a_{wopt} and a_{wmax}.

The four species considered in this study are characterised by significantly different estimated values for a_{wmin} (see Table 1). The estimated value of a_{wmin} 0.85 obtained for *C. cladosporioides* is consistent with literature data: 0.88 (Magan and Lacey, 1984), 0.84 (Grant et al., 1989), 0.845 (Rowan et al., 1999) and 0.86 a_w (Hocking et al., 1994). Similarly, the estimated value of a_{wmin} 0.88 obtained for *Alt. alternata* is in good agreement with other published data, 0.85 (Panasenko, 1967), 0.89 (Grant et al., 1989), 0.88 (Magan and Lacey, 1984) and 0.885 a_w (Rowan et al., 1999).

The minimum water activity for growth of *A. flavus* ranges from 0.81 to 0.95 (Gibson et al., 1994) depending on the humectant and the temperature. For conditions similar to those of our experiments, a_{wmin} has been reported as 0.85 for *A. flavus*, grown at temperatures ranging from 22°C and 33°C using glycerol as humectant (Holmquist et al., 1983) and at

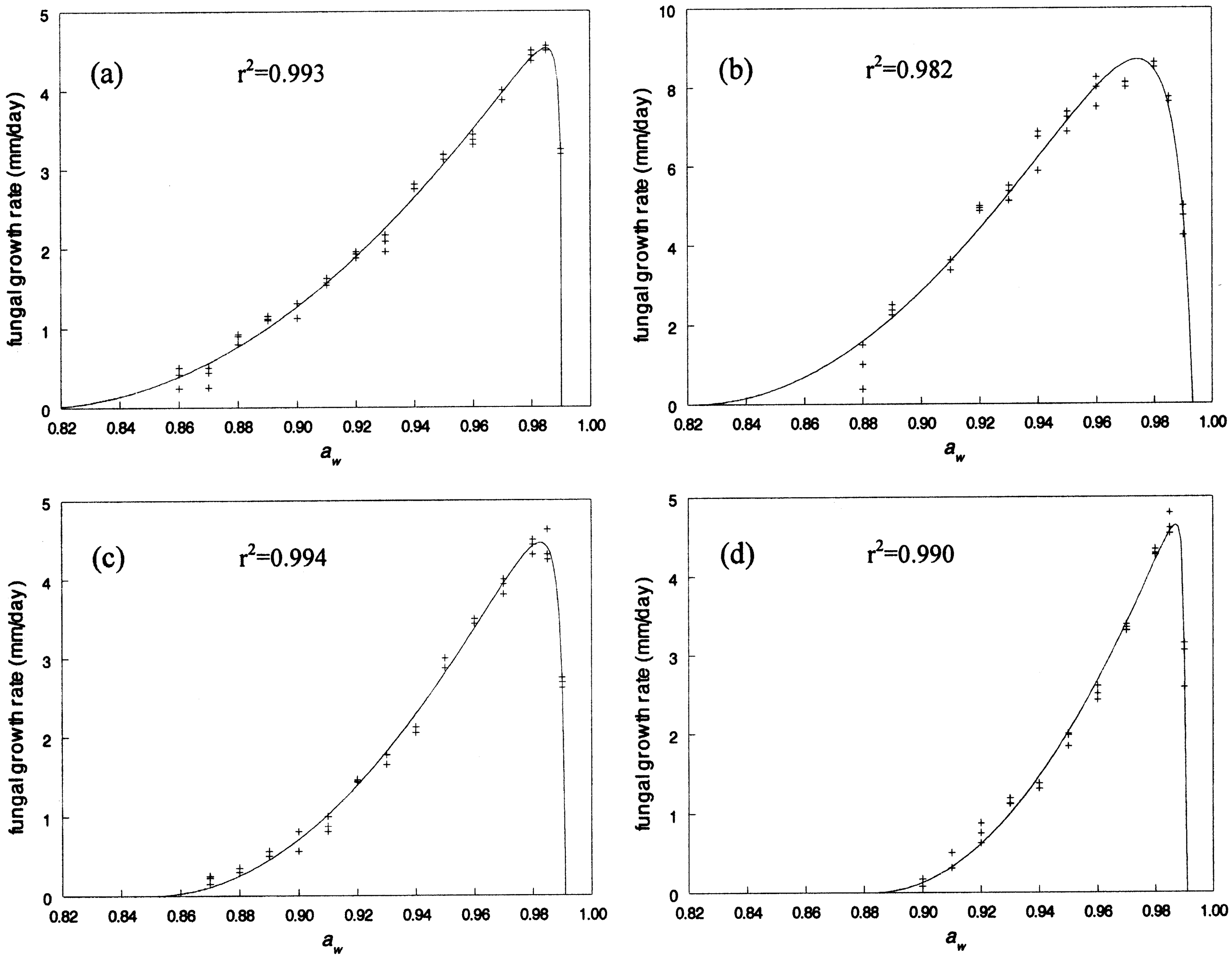

Fig. 1. Experimental plots (+) and the Rosso-type model (Eq. (2)) (−), growth rate vs. a_w for *P. chrysogenum* (a), *A. flavus* (b), *C. cladosporioides* (c) *Alt. alternata* (d) grown in PDA medium, pH 5.8 at their respective optimal temperatures of 25 ± 1°C (a) and (d); 26 ± 1°C (c).

30°C using a mixture of glucose and glycerol as humectant (Wheeler et al., 1988). Lower values for a_{wmin} have been reported also, 0.78 at 33°C (Ayerst, 1969) and 0.81 a_w at 30°C (Pitt and Miscamble, 1995).

For *P. chrysogenum*, Hocking and Pitt (1979) reported 0.78 a_{wmin} at 25°C, as compared to 0.79 at 25°C (Armolik and Dickson, 1956) and 0.81 a_w at 23°C (Mislivec and Tuite, 1970). The model estimation of 0.81 agrees well with these published data.

The model estimations have been compared to the experimental a_{wmin} defined as the water activity at which no growth can be observed after 3 weeks of incubation. Experimental values of 0.83, 0.87, 0.86, and 0.88 have been found for *P. chrysogenum*, *A. flavus*, *C. cladosporioides* and *Alt. alternata*, respectively. The experimental results are in accordance with the estimated values for *C. cladosporioides* and *Alt. alternaria*. For *P. chrysogenum* and *A. flavus*, the estimated values are significantly less than the experimental ones. It has been suggested that 3 weeks of incubation is not enough to determine whether growth occurs. For example, Pitt and Hocking (1977) incubated mould spores for 100 days and Snow (1949) found growth of *Eurotium echnulatum* after 3 years. In our experiments, no growth of *P. chrysogenum* was observed after 3 weeks at 0.83 a_w, and this was taken as the lower a_w limit for growth.

Water activity cannot exceed the value of 1, which is obtained for pure water. Slightly lower values were obtained as a_{wmax} using Eq. (2). Therefore, values of 0.99 could represent a media where no growth can be observed, but different from pure water. Nevertheless, by substituting a_{wmax} for 1 in Eq. (2) no significant alteration in the estimation of the other parameters (e.g. μ_{opt}, a_{wopt} and a_{wmin}) was observed. Therefore, for convenience the maximum water activity (a_{wmax}) for growth of these moulds can be set to 1.

Comparisons between experimental data and the model estimates are shown in Fig. 1. Excellent regression coefficients greater than 0.990 are observed for *P. chrysogenum*, *C. cladosporioides* and *Alt. alternata* suggesting the validity of this model for describing the influence of water activity on mould growth. The regression coefficient of 0.982 obtained for *A. flavus* can be explained partly by a discrepancy in the data obtained at a_w 0.88. Should these data be omitted the regression coefficient increases to 0.987, without any significant alteration in the parameter estimations. This result suggests that the difference in the estimated and the observed values for a_{wmin} is not due to the data obtained at a_w 0.88.

Although the influence of environmental factors on microbial growth can be explained by a variety of models, there is a need for standardising protocols. Through this example, the use of the model of Rosso established to represent the relationship between temperature and bacterial growth rate has been extended to the relationship between water activity and the growth of the four moulds considered here. This was possible because the shapes of the curves μ vs. temperature and μ vs. water activity are similar. Therefore, the shape of the curve should determine the choice of the model rather than the organism type or the environmental parameter.

References

Alber, S.A., Schaffner, D.W., 1992. Evaluation of data transformations used with the square root and Schoolfield models for predicting bacterial growth rate. Appl. Environ. Microbiol. 58, 3337–3342.

Armolik, N., Dickson, J.G., 1956. Minimum humidity requirements for germination of conidia of fungi associated with storage of grain. Phytopathology 46, 462–465.

Ayerst, G., 1969. The effects of moisture and temperature on growth and spore germination in some fungi. J. Stored Prod. Res. 5, 127–141.

Buchanan, R.L., Phillips, J.G., 1990. Response surface model for predicting the effects of temperature, pH, sodium chloride content, sodium nitrate concentration and atmosphere on the growth of *Listeria monocytogenes*. J. Food Prot. 53, 370–376.

Cuppers, H.G.A.M., Oomes, S., Brul, S., 1997. A model combining effects of temperature and salt concentration on the growth rate of food spoilage molds. Appl. Environ. Microbiol. 63, 3764–3769.

Dantigny, P., 1998. Dimensionless analysis of the microbial growth rate dependence on sub-optimal temperatures. J. Ind. Microbiol. Biotechnol. 21, 215–218.

Gervais, P., Bensoussan, M., Grajek, W., 1988. Water activity and water content: comparative effects on the growth of *Penicillium roquefortii* on a solid substrate. Appl. Microbiol. Biotechnol. 27, 389–392.

Gibson, A.M., Hocking, A.D., 1997. Advances in the predictive modelling of fungal growth in food. Trends Food Sci. Technol. 8, 353–358.

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

Grant, C., Hunter, C.A., Flannigan, B., Bravery, A.F., 1989. The moisture requirements of molds isolated from domestic dwellings. Int. Biodeterior. 25, 259–284.

Hocking, A.D., Pitt, J.I., 1979. Water relations of some *Penicillium* species at 25°C. Trans. Br. Mycol. Soc. 73, 141–145.

Hocking, A.D., Miscamble, B.F., Pitt, J.I., 1994. Water relations of *Alternaria alternata*, *Cladosporium cladosporioides*, *Cladosporium sphaerospermum*, *Curvularia lunata* and *Curvularia pallescens*. Mycol. Res. 98, 91–94.

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*. J. Food Sci. 48, 778–782.

Lacey, J., 1989. Pre- and post-harvest ecology of fungi causing spoilage of foods and other stored products. Filamentous Fungi in Foods and Feeds. Soc. Appl. Bacteriol. Symp. Ser. vol. 18, pp. 11–25.

Magan, N., Lacey, J., 1984. Effect of temperature and pH on water relations of field and storage fungi. Trans. Br. Mycol. Soc. 82, 71–81.

McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., Olley, J., Putro, S., Ratkowski, D.A., 1987. A model for combined effect of temperature and salt concentration/water activity on the growth rate of *Staphylococcus xylosus*. J. Appl. Bacteriol. 62, 453–550.

Mislivec, P.B., Tuite, J., 1970. Temperature and relative humidity requirements of species of *Penicillium* isolated from yellow dent corn. Mycologia 62, 75–88.

Panasenko, V.T., 1967. Ecology of microfungi. Bot. Rev. 33, 189–215.

Pitt, J.I., Hocking, A.D., 1977. Influence of solute and hydrogen ion concentration on the water relations of some xerophilic fungi. J. Gen. Microbiol. 101, 35–40.

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

Ratkowski, D.A., Lowry, R.K., McMeeking, T.A., Stokes, A.N., Chandler, R.E., 1983. Model for bacterial growth rate throughout the entire biokinetic temperature range. J. Bacteriol. 154, 1222–1226.

Rosso, L., Lobry, J.R., Bajard, S., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J. Theor. Biol. 162, 447–463.

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. Appl. Environ. Microbiol. 61, 610–616.

Rowan, N.J., Johnstone, C.M., McLean, R.C., Anderson, J.G., Clarke, J.A., 1999. Prediction of toxigenic fungal growth in buildings by using a novel modelling system. Appl. Environ. Microbiol. 65, 4814–4818.

Samson, R., Hoeckstra, E.S., Frisvad, J.C., Filtenborg, O., 1995. Introduction to Food-Borne Fungi. Centrallbureau voor Schimmelcultures, Baarn, Netherlands.

Scott, W.J., 1957. Water relations of food spoilage microorganisms. Adv. Food Res. 7, 83–127.

Snow, D., 1949. The germination of mould spores at controlled humidities. Ann. Appl. Biol. 36, 1–13.

Trinci, A.P.J., 1969. A kinetic study of the growth of *Aspergillus nidulans* and other fungi. J. Gen. Microbiol. 57, 11–24.

Wheeler, K.A., Hocking, A.D., Pitt, J.I., 1988. Water relations of some *Aspergillus* species isolated from dried fish. Trans. Br. Mycol. Soc. 91, 631–637.

Zwietering, J.T., Koos, J.T., Hasenack, B.E., Wit, J.C., Van't Riet, K., 1991. Modelling of bacterial growth as a function of temperature. Appl. Environ. Microbiol. 57, 1094–1101.

Zwietering, M.H., Wit, J.C., Cuppers, H.G.A.M., Van't Riet, K., 1994. Modelling of bacterial growth with shifts in temperature. Appl. Environ. Microbiol. 60, 204–213.