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Modeling growth of three bakery product spoilage molds as a function of water activity, temperature and pH

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ABSTRACT

The objective of this study was to quantify the effect of water activity, pH and storage temperature on the growth of *Eurotium repens*, *Aspergillus niger* and *Penicillium corylophilum*, isolated from spoiled bakery products. Moreover, the behaviors of these three mold species were compared to assess whether a general modeling framework may be set and re-used in future research on bakery spoilage molds. The mold growth was modeled by building two distinct Gamma-type secondary models: one on the lag time for growth and another one on the radial growth rate. A set of 428 experimental growth curves was generated. The effect of temperature (15–35 °C), water activity (0.80–0.98) and pH (3–7) was assessed. Results showed that it was not possible to apply the same set of secondary model equations to the three mold species given that the growth rate varied significantly with the factors pH and water activity. In contrast, the temperature effect on both growth rate and lag time of the three mold species was described by the same equation. The equation structure and model parameter values of the Gamma models were also compared per mold species to assess whether a relationship between lag time and growth rate existed. There was no correlation between the two growth responses for *E. repens*, but a slight one for *A. niger* and *P. corylophilum*. These findings will help in determining bakery product shelf-life and guiding future work in the predictive mycology field.

1. Introduction

Mold spoilage of bakery products causes great economic losses, estimated globally to one to three percent volume (Malkki and Ravha, 1978). The molds frequently involved are *Penicillium*, *Aspergillus*, *Eurotium* and *Wallemia* species (Dantigny et al., 2005b; Vytrasova et al., 2002).

Mold spoilage results from the contamination of a product with fungal spores, which further, if the conditions are appropriate, may germinate and form a visible mycelium before the time of consumption (Dagnas and Membré, 2013; Gougouli et al., 2011; Horner and Anagnostopoulos, 1973). Generally, mold growth can be prevented by the application of the hurdle technology concept (Leistner, 2000). Among the preservative factors water activity (a_w) is often reported as the most effective one on inhibiting mold growth (Pitt and Hocking, 1977; Sautour et al., 2001a) given that a_w decrement leads to a decrease of the speed of both germination and mycelium growth until a minimum where neither germination nor growth occurs. Beside a_w, temperature is recognized to limit mold growth on its own or in combination with a_w (Abellana et al., 1999; Marín et al., 1996). On the other hand, the role of pH in mold growth limitation has never been clearly established, at least in the range of mostly bakery product formulation, i.e. range of pH 3.5–7.5 (Sautour et al., 2001b; Wheeler et al., 1991).

To assess quantitatively the effect of preservative factors on mold growth, the use of predictive microbiology models is valuable. In the recent past, various types of primary models have been developed to describe either germination or mycelium growth kinetics under various conditions (Dagnas and Membré, 2013; Dantigny et al., 2005a). In addition, several types of secondary models describing the effect of the preservative factors a_w, temperature and pH on these kinetics have been suggested (Garcia et al., 2009). Among them, the Gamma-type models have been widely applied to mycelium radial growth rate, on various mold species (e.g., *Aspergillus flavus*, *Aspergillus candidus*, *Aspergillus ochraceus*, *Aspergillus parasiticus*, *Aspergillus carbonarius*, *Penicillium expansum*, *Penicillium glabrum*, *Botrytis cinerea* and *Monascus ruber*) and various food products (e.g., corn, grape, peanut, aromatized water and table olives) (Astoreca et al., 2012; Garcia et al., 2011; Huchet et al., 2013; Judet-Correia et al., 2010; Nevarez et al., 2009; Panagou et al., 2003; Tassou et al., 2007). Concerning the lag time for growth only few applications of the Gamma-type model structure have been described (Gougouli et al., 2011; Gougouli and Koutsoumanis, 2010; Huchet et al., 2013; Marín et al., 2009). This time includes the germination time and is driven by the effect of environmental factors (Gougouli and Koutsoumanis, 2012, 2013). When it has been modeled, this lag time was described as a function of only one preservative factor, either

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the a_w (Marín et al., 2009) or the temperature (Gougouli et al., 2011; Gougouli and Koutsoumanis, 2010).

The first objective of this study was to develop predictive microbiology models to assess the effect of a_w, temperature, and, if necessary, pH on growth (lag time for growth and radial growth rate) of three molds isolated from spoiled bakery products. Once the models are developed and validated, they will enable to calculate the probability of having mold growth up to a visible mycelium before the time of consumption, and subsequently to determine the bakery product shelf-life (Dagnas and Membré, 2013). The models were developed for *Aspergillus niger*, *Penicillium corylophilum* and *Eurotium repens*, mold species frequently encountered in bakery products.

The second objective of this study was to assess whether the same model structure (i.e. same equation) could be applied to the three mold species studied here, meaning that the three species would have the same response to the hurdle factor combination, differing only by the response intensity or the range of parameter values (i.e. different model parameter estimates). If this assumption is right, that may simplify the future research in the field of mold spoilage in food as a general model framework will be in place.

The third objective of this study was to compare the secondary model outputs (equation structure and model parameter estimates) for both radial growth rate and lag time for growth. Indeed the aim was to assess if the duration of the lag time could be related to the radial growth rate in terms of water activity effect, temperature effect or pH effect (e.g., same cardinal values, proportionality between both kinetic parameters). If a relationship is found, future development in the predictive mycology area will be simplified: it will be possible to deduce the lag time from the mycelium radial growth rate, or *vice versa*.

2. Materials and methods

2.1. Fungal strains

One strain of three major fungal species responsible for the spoilage of bakery products was isolated, identified using phenotypic and genotypic methods and provided by the Culture Collection of Université de Bretagne Occidentale (UBOCC, Plouzané, France). The strains were *Eurotium repens* UBOCC-A-112075, *Aspergillus niger* UBOCC-A-112064 and *Penicillium corylophilum* UBOCC-A-112081. They were maintained and subcultured bimonthly on Malt Extract Agar (MEA, AES Chemunex, Bruz, France).

2.2. Spore suspension preparation

The three strains were grown separately on MEA media for 7 days at 25 °C to obtain heavily sporulating cultures. Spores suspensions were then prepared by washing the cultures with sterile distillated water containing 0.01% (vol/vol) Tween® 80 (Merck KGaA, Darmstadt, Germany) (Dantigny et al., 2006). The spore concentration of each suspension was assessed using a Malassez counting chamber and adjusted if necessary to 10^6 spores/mL with appropriate dilutions. To minimize the potential impact of the water activity change between the suspension and the growth medium (as suggested by Nanguy et al. (2010)), the final suspensions were used as quickly as possible (less than a few minutes) for inoculation.

2.3. Experimental design

A 5-level Latin Square design was set up to study the effect of water activity (a_w), temperature (T) and pH on mold growth within 25 combinations of experimental conditions per strain studied. This design allows reducing the number of experiments while keeping an acceptable level of accuracy and reliability in estimating the parameters (Mertens et al., 2012; Van Derlinden et al., 2013). The a_w was set at 0.80, 0.85, 0.90, 0.94 and 0.98, the temperature at 15, 21, 25, 30 and 35 °C and pH at 3, 4, 5, 6 and 7. The Latin Square design was carried out after a randomization of the factor level order. Three plates were used per combination (=75 experiments) for each of the three strains studied (=225 experiments). The experimental design was carried out twice (one repetition), which means a set of 450 experiments. Moreover, 22 mold growth experiments were found contaminated by unexpected molds and then discarded. Consequently, the final set of data contained 428 experimental mold growth curves. The three plates used for each experimental combination of one repetition corresponded to the repeatability of the experimental set up, the duplicates (the whole experimental design repeated once) to the reproducibility.

2.4. Media preparation, inoculation and incubation

All experiments were performed on MEA Petri dishes. Water activity values were adjusted by replacing a part of the water by glycerol during media preparation as recommended by Hocking and Pitt (1980). To determine the amount of glycerol added, a standard curve describing the water activity of the MEA medium as function of the glycerol part (in %, wt/wt) was built (linear relationship, $R^2 = 0.998$, data not shown). Water activity measurements were performed with an a_w meter (*Lab*MASTER-a_w, Novasina AG, Lachen, Switzerland). During media preparation pH values were adjusted by adding drops of either a 0.5 M HCl solution or a 0.5 M NaOH solution until the required value was reached. The pH was measured continuously for this operation with a pH meter (MP225, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) equipped with a glass electrode. For pH 5, 6 and 7 adjustments were carried out before sterilization (the pH value was verified after the sterilization). For pH 3 and 4, adjustments were made after sterilization in a medium cooled down to 45 °C to enable its further solidification. After sterilization process (121 °C during 15 min) adjusted MEA were poured (20 mL) into identified 90 mm diameter sterile Petri dishes. For each strain, 2×10^3 spores (2 μL of the 10^6 spores/mL suspension) were inoculated centrally.

All the inoculated dishes were then sealed with Parafilm® to avoid dehydration and incubated at the required temperatures in low temperature programmable incubators (MIR 154, Sanyo Electric Co., Ltd., Osaka, Japan). The Petri dishes at the same a_w and temperature were placed together with a mixture water/glycerol in sealed polyethylene bag to keep a better equilibrium.

2.5. Growth measurement and primary modeling

Each plate was checked daily for mold growth during 35 days (experimental duration chosen according to the shelf-life of the bakery products studied, i.e. 20–30 days). In case of growth, colony diameters were measured in two directions at right angles to each other with a digital caliper until the colony reached a limit value of 25 mm. Indeed, after 25 mm it was noticed in some conditions, a radial growth rate slow-down, phenomenon also observed by Baranyi et al. (2014).

Radius of the colonies were then plotted against the time and fitted to a primary model with latency (Buchanan et al., 1997) (Eq. (1)) to estimate the lag time before mycelium growth (days) and the mycelium radial growth rate (mm/day). The non-linear regression of the primary model was performed using the nls function from the R software (R-Development-Core-Team, 2012). The results were analyzed furthermore using the nlstools package (Baty and Delignette-Muller, 2012) implemented in R.

$$R_{(t)} = \begin{cases} R_0, & t \le \lambda \\ R_0 + \mu.(t-\lambda), & t > \lambda \end{cases} \quad (1)$$

In Eq. (1), t is the time (day), $R_{(t)}$ (mm) is the colony radius at time t, R_0 (mm) is the initial colony radius (equal to the radius of the spore suspension inoculation drop, 3 mm), λ is the lag time before mycelium growth (days), and μ is the mycelium radial growth rate (mm radius/day).

When no growth was observed within 35 days (139 growth curves of 428), the lag time was defined as longer than 35 days ($\lambda > 35$ days) and the radial growth rate as zero ($\mu = 0$). When the mycelium growth was too fast (12 growth curves of 428), there was not enough data to determine accurately the growth kinetics. In such a case, the growth rate was defined as higher than the highest growth rate calculated for the same mold strain, μ_{max}. Likewise, the lag time was defined as lower than the lowest lag time calculated for the same mold strain, λ_{min}. These data were analyzed as left or right censored data: left for $1/\lambda < 1/35$ days, right for $1/\lambda > 1/\lambda_{min}$ and for $\mu > \mu_{max}$.

2.6. Secondary modeling

The 428 estimated μ and λ parameters were then expressed as function of a_w and temperature using Gamma-concept models firstly introduced by Zwietering et al. (1993). The radial growth rate, μ, was modeled after a square root transformation (Eq. (2)), and the inverse of the lag time, $1/\lambda$, was modeled also after a square root transformation (Eq. (3)) allowing the stabilization of the variance as recommended by Dantigny and Bensoussan (2008).

$$\sqrt{\mu_K} = \sqrt{\mu_K^{opt}} \cdot \sqrt{\left(\gamma_K^{\mu}(a_w) \cdot \gamma_K^{\mu}(T)\right)} + \varepsilon_\mu \quad (2)$$

$$\sqrt{1/\lambda_K} = \sqrt{1/\lambda_K^{opt}} \cdot \sqrt{\left(\gamma_K^{\lambda}(a_w) \cdot \gamma_K^{\lambda}(T)\right)} + \varepsilon_\lambda \quad (3)$$

where K represents the strains ($K = 1, 2$ and 3 for *E. repens*, *A. niger* and *P. corylophilum* respectively), and μ^{opt} and λ^{opt} are the values of μ and λ at optimal a_w and temperature (T). The $\gamma(.)$ terms represent the effect of a_w and temperature on μ and λ.

The two models had a hierarchical structure: the terms ε_μ and ε_λ representing the residual errors were assumed to be identical whatever the species. On the other hand, the residual errors were not further broken down into repeatability and reproducibility error terms.

For each strain and for both kinetic responses (radial growth rate and lag time for growth), several cardinal model structures were tested and their parameters estimated through Bayesian inference. The final estimated parameters were T_{min}, T_{opt}, T_{max}, $a_{w\ min}$, $a_{w\ opt}$, $\sqrt{\mu}^{opt}$ and $\sqrt{1/\lambda}^{opt}$. The parameter $a_{w\ max}$ was fixed to the value 1 as suggested by Sautour et al. (2001a) since the three mold species studied have been reported to grow at a_w higher than 0.99 (Andrews and Pitt, 1987; Belĺı et al., 2004; Pitt and Hocking, 2009).

2.7. Bayesian inference

In Bayesian inference, the posterior probabilities are defined as proportional to prior probabilities times the likelihood of the data (Eq. (4)).

$$p(\text{parameters}/\text{data}) \propto P(\text{parameters}) \times P(\text{data}/\text{parameters}) \quad (4)$$

In other words Bayesian inference enables to integrate an a priori knowledge on the model parameters through prior probability distributions into the estimation process. Here, Bayesian inference was used to include information on *A. niger* T_{opt} and T_{max}, for which information was available (Cuppers et al., 1997; Gougouli et al., 2011; Gougouli and Koutsoumanis, 2010, 2012; Parra and Magan, 2004). The prior distributions for the three species parameters were chosen as normal distributions (Table 1). Normal distributions for the model parameters are often chosen in Bayesian statistics. The reason for this choice lies in the following statistical property: with a Normal likelihood and a normal prior, the posterior distribution is also a normal distribution (Marin and Robert, 2007). This property is an algebraic convenience; otherwise a difficult numerical integration may be necessary during the model inferring step.

Table 1
Prior probability distributions of the parameters estimated through the Bayesian inference process for both radial growth rate and lag time models. The distributions are presented as ~N(mean; variance).

	Eurotium repens	*Aspergillus niger*	*Penicillium corylophilum*
$a_{w\ min}$	$\sim\mathcal{N}(0.8; 100)$	$\sim\mathcal{N}(0.8; 100)$	$\sim\mathcal{N}(0.8; 100)$
$a_{w\ opt}$	$\sim\mathcal{N}(0.95; 100)$	$\sim\mathcal{N}(0.95; 100)$	$\sim\mathcal{N}(0.95; 100)$
$a_{w\ max}$	1[a]	1[a]	1[a]
T_{min}	$\sim\mathcal{N}(5; 1000)$	$\sim\mathcal{N}(5; 1000)$	$\sim\mathcal{N}(5; 1000)$
T_{opt}	$\sim\mathcal{N}(27; 1000)$	$\sim\mathcal{N}(34; 2.341)$	$\sim\mathcal{N}(27; 1000)$
T_{max}	$\sim\mathcal{N}(40; 1000)$	$\sim\mathcal{N}(45.5; 1.638)$	$\sim\mathcal{N}(40; 1000)$
$\sqrt{\mu_K^{opt}}$	$\sim\mathcal{N}(3; 1000)$	$\sim\mathcal{N}(3; 1000)$	$\sim\mathcal{N}(3; 1000)$
$\sqrt{1/\lambda_K^{opt}}$	$\sim\mathcal{N}(2; 1000)$	$\sim\mathcal{N}(2; 1000)$	$\sim\mathcal{N}(2; 1000)$
ε_μ and ε_λ	$\sim\mathcal{N}(0; \sigma^2)$ with $\sigma\sim\mathcal{U}(0.1; 10)$	$\sim\mathcal{N}(0; \sigma^2)$ with $\sigma\sim\mathcal{U}(0.1; 10)$	$\sim\mathcal{N}(0; \sigma^2)$ with $\sigma\sim\mathcal{U}(0.1; 10)$

[a] Parameters not estimated but fixed.

Bayesian inference was also chosen as it facilitates the integration of censored data (139 + 12 lag time data of 428; 12 growth rate data of 428). Indeed, Eq. (4) was modified as follows (Mitra, 2013):

Right censored:

$$P(\text{parameters}/\text{data}) \propto P(\text{paramters}) \times \prod_{i \in U} f(Y_i) \times \prod_{i \in C} \{1 - F(Y_i)\} \quad (5)$$

Left censored:

$$P(\text{parameters}/\text{data}) \propto P(\text{parameters}) \times \prod_{i \in U} f(Y_i) \times \prod_{i \in C} F(Y_i) \quad (6)$$

Where Y is the response studied ($\sqrt{\mu}$ or $\sqrt{1/\lambda}$), $f(Y)$ is the probability distribution function of Y, $F(Y)$ is the cumulative distribution function and U and C denote the sets that contain the uncensored and censored data, respectively.

Computing these equations (from 4 to 6) is often analytically impossible. A variety of methods have been developed to carry out a modeling approach based on Bayesian inference. One of the most popular ones is the Markov Chain Monte Carlo technique in which a Markov Chain is used to sample for the posterior distributions (Dakins et al., 1996; Patwardhan and Small, 1992). The solving process, where the posterior distributions of the model parameters are computed at each iteration, was run with the WinBUGS package (Lunn et al., 2000). To check the convergence of the iteration process, visual analyses (history function and Gelman and Rubin diagnostic) of three independent chains were performed. Twenty thousand iterations were run; the first 10,000 iterations were eliminated (burn-in period). Moreover, the pairwise parameter correlations were calculated and their scatter plots were generated.

2.8. Goodness of fit and choice of the Gamma terms

For both radial growth rate and lag time models, several Gamma terms were tested: for temperature and a_w, cardinal model equations with $n = 1$ or $n = 2$ (Rosso et al., 1995), also for a_w an equation adapted from Zwietering et al. (1996). The first criterion to decide if a Gamma term was potentially suitable was the convergence after 20,000 iterations. If there was no convergence, the Gamma term was discarded. Next, a visual inspection of the residual errors (i.e. observed data minus fitted data) was carried out. In case of bias, the Gamma term was discarded. Finally, both standard deviation of the model errors (σ_μ and σ_λ) and the deviance information criteria (DIC_μ and DIC_λ) were calculated and compared. The standard deviation of the model error also called the root mean square error shows the average discrepancy between observed data and their fitted values and is a measure of

goodness-of-fit for regression models (Ratkowsky, 2004). The DIC is a model comparison criterion (generalization of the Akaike information criterion) that combines Bayesian measure of fit with a measure of model parsimony (Berg et al., 2004). The Gamma term leading to the smallest values of these two statistical criteria was kept in the model.

2.9. Assessing the impact of pH

Initially, the pH effect on mold growth responses was assessed on the three molds with a pH Gamma term written as follows:

$$\gamma_K^{\mu}(\mathrm{pH}) = \begin{cases} 0, & \mathrm{pH} \le \mathrm{pH}_{\min} \\ \dfrac{(\mathrm{pH}-\mathrm{pH}_{\max})(\mathrm{pH}-\mathrm{pH}_{\min})}{[(\mathrm{pH}-\mathrm{pH}_{\max})(\mathrm{pH}-\mathrm{pH}_{\min})]-\left(\mathrm{pH}-\mathrm{pH}_{\mathrm{opt}}\right)^2}, & \mathrm{pH}_{\min}<\mathrm{pH}<\mathrm{pH}_{\max} \\ 0, & \mathrm{pH} \ge \mathrm{pH}_{\max} \end{cases} \quad (7)$$

In Eq. (7), $\mathrm{pH}_{\mathrm{opt}} = \frac{\mathrm{pH}_{\min}+\mathrm{pH}_{\max}}{2}$.

However, with this Gamma term applied to the three strains in the two hierarchical models (Eqs. (2) and (3)) and even after 50,000 iterations, convergence of the Markov chains did not occur. Consequently, to check if the pH had an effect on either lag time for growth or radial growth rate, the residual errors calculated from the model without the pH Gamma term (Eqs. (2) and (3)) were analyzed graphically. In case of trend (i.e. indicating a potential pH effect), the hierarchical models were split into single-strain models on which the pH Gamma term was tested as described above.

3. Results

3.1. Primary modeling

On 428 growth experiments (450 minus 22 contaminated), 139 led to no growth within 35 days (μ set to zero and λ set to >35 days) and 289 led to growth within 35 days. In the latter μ and λ were estimated through primary modeling. The relative errors of the parameters, i.e. standard error divided by mean, were estimated to a mean of 3.3% (median = 2.4%) and 8.4% (median = 3.8%) for μ and λ, respectively.

3.2. Predictive models for radial growth rate and lag time

Among the Gamma terms tested, the ones presented in Tables 2 and 3 (Eqs. (8) to (12)) gave the best results regarding the statistical criteria (smallest errors and DIC) and were kept in the models.

The standard deviations of the residual errors were estimated to 0.31 (23% of the average response) and 0.16 (26% of the average response) for radial growth rate and lag time models, respectively. The parameters were accurately estimated (Tables 4 and 5) except the $T_{\min}$ for mycelium growth of *E. repens* and *P. corylophilum*, which showed a standard deviation higher than 3 °C. No structural correlation was found between the estimated parameters (Figs. S1 and S2). The fitted and observed values of radial growth rates and lag times were plotted as a function of a_w (Figs. 1 and 2, respectively), for the three molds.

3.3. Effect of pH

It was not possible to run the Markov Chain when a pH Gamma term was included in the hierarchical models neither for the growth rate nor for the lag time for growth. Then, to check if the pH might have an effect on one of these two responses, a further investigation was done. The residual errors of both models without pH Gamma terms (Eqs. (2) and (3)) were plotted versus the pH for each of the strain. While there was no bias for the lag time of any of the strain (data not shown), a slight structure of the residual error plot was noticed for *E. repens* growth rate model (Fig. 3A). To assess whether this effect was significant, a comparison between a growth rate model (Eq. (2)) limited to *E. repens*, completed with the pH Gamma term as described in Eq. (7) and a growth rate model (Eq. (2)) limited to *E. repens* without pH Gamma term was made. Results indicated a slight effect of pH on *E. repens* radial growth rate ($\sigma_\mu = 0.36$ and 0.42 for the model with pH and the model without pH term, respectively; DIC = 126 and 169 for the model with pH and the model without pH term, respectively). The parameter values associated with *E. repens* growth rate model (Eq. (2)) completed by the pH Gamma term (Eq. (7)) are provided in Table 6, the residual error plot is given in Fig. 3B.

3.4. Effect of temperature and a_w on the mycelium radial growth rate

The model structure describing the effect of a_w on *E. repens* radial growth rate (cardinal model, $n = 2$, Eq. (8) in Table 2) differed from the one applied to *A. niger* and *P. corylophilum* (equation adapted from Zwietering et al. (1996), Eq. (9) in Table 2) (Fig. 1). Regarding the storage temperature, the three molds showed a comparable pattern of mycelium growth described by a $n = 2$ cardinal model (Eq. (10) in Table 2). In terms of range of parameter values, although the $a_{w\ min}$ of the three strains were similar (ca. 0.79), *E. repens* had an $a_{w\ opt}$ value of 0.91, whereas *A. niger* and *P. corylophilum* showed the highest growth rates at the maximum a_w tested (i.e. 0.98). *A. niger* was able to grow at higher temperature than *E. repens* and *P. corylophilum* (Table 4). These two latter strains grew at temperature between 0 and 35 °C with an optimum temperature value estimated to 29.0 °C for *E. repens* and to 25.8 °C for *P. corylophilum*. On the other hand, *A. niger* had a $T_{\min}$, T_{opt} and $T_{\max}$ for mycelium growth of 7.0 °C, 34.9 °C and 45.7 °C, respectively.

Table 2
Gamma terms associated with the radial growth rate model (Eq. (2)).

Factor	Gamma term		Species on which the Gamma term was applied
a_w	$\gamma_K^{\mu}(a_w) = \begin{cases} 0, & a_w \le a_{w\ min} \\ \dfrac{(a_w-a_{w\ min})^2(a_w-a_{w\ max})}{(a_{w\ opt}-a_{w\ min})\left[(a_{w\ opt}-a_{w\ min})(a_w-a_{w\ opt})-(a_{w\ opt}-a_{w\ max})(a_{w\ opt}+a_{w\ min}-2a_w)\right]}, & a_{w\ min}<a_w<a_{w\ max} \\ 0, & a_w \ge a_{w\ max} \end{cases}$	(8)	*Eurotium repens*
	$\gamma_K^{\mu}(a_w) = \begin{cases} 0, & a_w \le a_{w\ min} \\ \left(\dfrac{a_w-a_{w\ min}}{1-a_{w\ min}}\right)^2, & a_{w\ min} \le a_w < 1 \end{cases}$	(9)	*Aspergillus niger* *Penicillium corylophilum*
T	$\gamma_K^{\mu}(T) = \begin{cases} 0, & T \le T_{\min} \\ \dfrac{(T-T_{\min})^2(T-T_{\max})}{(T_{opt}-T_{\min})\left[(T_{opt}-T_{\min})(T-T_{opt})-(T_{opt}-T_{\max})(T_{opt}+T_{\min}-2T)\right]}, & T_{\min}<T<T_{\max} \\ 0, & T \ge T_{\max} \end{cases}$	(10)	*E. repens* *A. niger* *P. corylophilum*

Table 3
Gamma terms associated with the lag time for growth model (Eq. (3)).

Factor	Gamma term		Species on which the Gamma term was applied
a_w	$\gamma_K^{\lambda}(a_w) = \begin{cases} 0, & a_w \le a_{w\ min} \\ \left(\frac{a_w - a_{w\ min}}{1 - a_{w\ min}}\right)^2, & a_{w\ min} \le a_w < 1 \end{cases}$	(11)	*Eurotium repens* *Aspergillus niger* *Penicillium corylophilum*
T	$\gamma_K^{\lambda}(T) = \begin{cases} 0, & T \le T_{min} \\ \frac{(T-T_{min})^2 (T-T_{max})}{(T_{opt}-T_{min})\left[(T_{opt}-T_{min})(T-T_{opt}) - (T_{opt}-T_{max})(T_{opt}+T_{min}-2T)\right]}, & T_{min} < T < T_{max} \\ 0, & T \ge T_{max} \end{cases}$	(12)	*E. repens* *A. niger* *P. corylophilum*

3.5. Effect of temperature and a_w on the lag time for growth

The same model structure was applied to *E. repens*, *A. niger* and *P. corylophilum* lag times, for describing in one hand the a_w effect (Eq. (11) in Table 3), and on the other hand the temperature effect (Eq. (12) in Table 3). Lag times for mycelium growth were relatively short (0.5–3 days) near to a_w of 0.98 and increased with the decrease of a_w until the $a_{w\ min}$ values were reached (Fig. 2). *E. repens* had the lowest $a_{w\ min}$ with a value of 0.76 (Table 5). *A. niger* and *P. corylophilum* were comparable with an $a_{w\ min}$ around 0.80. *E. repens* had the narrowest range of temperature for the lag time with T_{min}, T_{opt} and T_{max} estimated to 12.8, 25.4 and 35.3 °C, respectively. *P. corylophilum* showed similar cardinal temperature values even if the range was slightly greater (T_{min} and T_{max} estimated to 9.8 and 37.0 °C, respectively). *A. niger* showed the largest range of cardinal temperature values with 9.0, 37.4 and 44.5 °C as minimum, optimum and maximum, respectively.

3.6. Comparison between radial growth rate and lag time for growth

For *E. repens*, the a_w Gamma terms were different for growth rate and lag time, meaning that overall, these both growth responses were not correlated. These findings were confirmed by observing the raw data (Fig. 4A).

On the other hand the same model structure was applied for both growth responses of *A. niger* and *P. corylophilum*. Consequently, a deeper analysis of the cardinal model parameter values was necessary to conclude whether a proportional relationship between growth rate and lag time exists. For *A. niger*, the cardinal parameter values (related to a_w and temperature) for lag time and for radial growth rate were equivalent (Tables 4 and 5), while they were somewhat different for *P. corylophilum*. Indeed, for *P. corylophilum* T_{min} was estimated to 0.9 and 9.8 °C for radial growth rate model and lag time for growth model, respectively. However, this difference has to be interpreted with care as the parameter standard deviations are large (from 2 to 5 °C). In addition to comparing the model parameters, the observation of the raw data indicated a slight proportional relationship between $\sqrt{\mu}$ and $\sqrt{1/\lambda}$ (Fig. 4B and C), the product μ times λ being 6.7 with a standard deviation of 3.7 for *A. niger* and 3.3 (standard deviation 1.6) for *P. corylophilum*.

4. Discussion

Original data on *E. repens*, *A. niger* and *P. corylophilum* growth have been collected. The experimental period of 35 days was set accordingly to the shelf-life of the bakery products studied (20–30 days). Then, predictive models have been developed for two responses (lag time for mycelium growth and mycelium radial growth rate), two factors (a_w and temperature) and three mold species with satisfactory results: small residual errors, parameters estimated accurately and no correlation between parameters. Moreover, the parameter values were compared with those reported in the literature (Table 7). For *A. niger*, the results were in agreement for both temperature and water activity parameter values. For *P. corylophilum* there was not enough data to reach a definitive conclusion. Concerning *E. repens*, temperature parameter estimates obtained in our study matched those reported in the literature. The difference found for $a_{w\ min}$ may be explained by the different food matrices from which the strains were isolated. For example, in our study the strain was isolated from bakery product (a_w 0.85) while in Andrews and Pitt (1987) the strain studied came from dried fish (a_w 0.65–0.79).

The advantages of having predictive models based on a Gamma model structure have been already largely mentioned in the literature and are (i) parsimony and interpretability of the model parameters, and (ii) possibility to complete the model by adding the effect of an extra preservative factor without breaking down the existing model structure. The Gamma type models have already been applied

Table 4
Estimated parameters of the radial growth rate model (Eq. (2)).

			Mean	sd	Lower 95% CI	Upper 95% CI
Temperature (°C)	T_{min}	*Eurotium repens*	0.6	3.2	−6.5	6.0
		Aspergillus niger	7.0	1.5	3.8	9.6
		Penicillium corylophilum	0.9	5.0	−9.6	10.1
	T_{opt}	*E. repens*	29.0	1.1	28.9	31.6
		A. niger	34.9	1.0	33.0	37.1
		P. corylophilum	25.8	1.4	23.3	28.5
	T_{max}	*E. repens*	35.1	0.1	35.0	35.3
		A. niger	45.7	1.2	43.2	48.1
		P. corylophilum	35.8	0.4	35.3	36.7
Water activity	$a_{w\ min}$	*E. repens*	0.789	3.13×10^{-3}	0.783	0.795
		A. niger	0.795	3.49×10^{-3}	0.788	0.802
		P. corylophilum	0.802	7.43×10^{-3}	0.788	0.817
	$a_{w\ opt}$	*E. repens*	0.914	3.41×10^{-3}	0.908	0.921
$\sqrt{\mu^{opt}}$ (mm/day)$^{0.5}$		*E. repens*	2.03	0.06	1.92	2.17
		A. niger	3.71	0.13	3.47	3.98
		P. corylophilum	1.95	0.08	1.80	2.10
σ_μ			0.31	0.01	0.29	0.33

Table 5
Estimated parameters of the lag time for growth model (Eq. (3)).

			Mean	sd	Lower 95% CI	Upper 95% CI
Temperature (°C)	T_{min}	*Eurotium repens*	12.8	1.0	10.6	14.3
		Aspergillus niger	9.0	0.9	7.1	10.7
		Penicillium corylophilum	9.8	2.2	4.8	13.3
	T_{opt}	*E. repens*	25.4	0.5	24.6	26.6
		A. niger	37.4	1.0	35.6	39.5
		P. corylophilum	25.7	0.7	24.7	27.2
	T_{max}	*E. repens*	35.3	0.1	35.1	35.7
		A. niger	44.5	1.3	41.9	47.0
		P. corylophilum	37.0	0.5	36.2	38.1
Water activity	$a_{w\ min}$	*E. repens*	0.763	9.45×10^{-3}	0.743	0.780
		A. niger	0.801	3.71×10^{-3}	0.793	0.809
		P. corylophilum	0.811	6.15×10^{-3}	0.799	0.823
$\sqrt{1/\lambda^{opt}}$ $(1/day)^{0.5}$		*E. repens*	1.05	0.04	0.98	1.12
		A. niger	2.00	0.10	1.83	2.20
		P. corylophilum	1.26	0.04	1.18	1.34
σ_λ			0.16	0.01	0.14	0.17

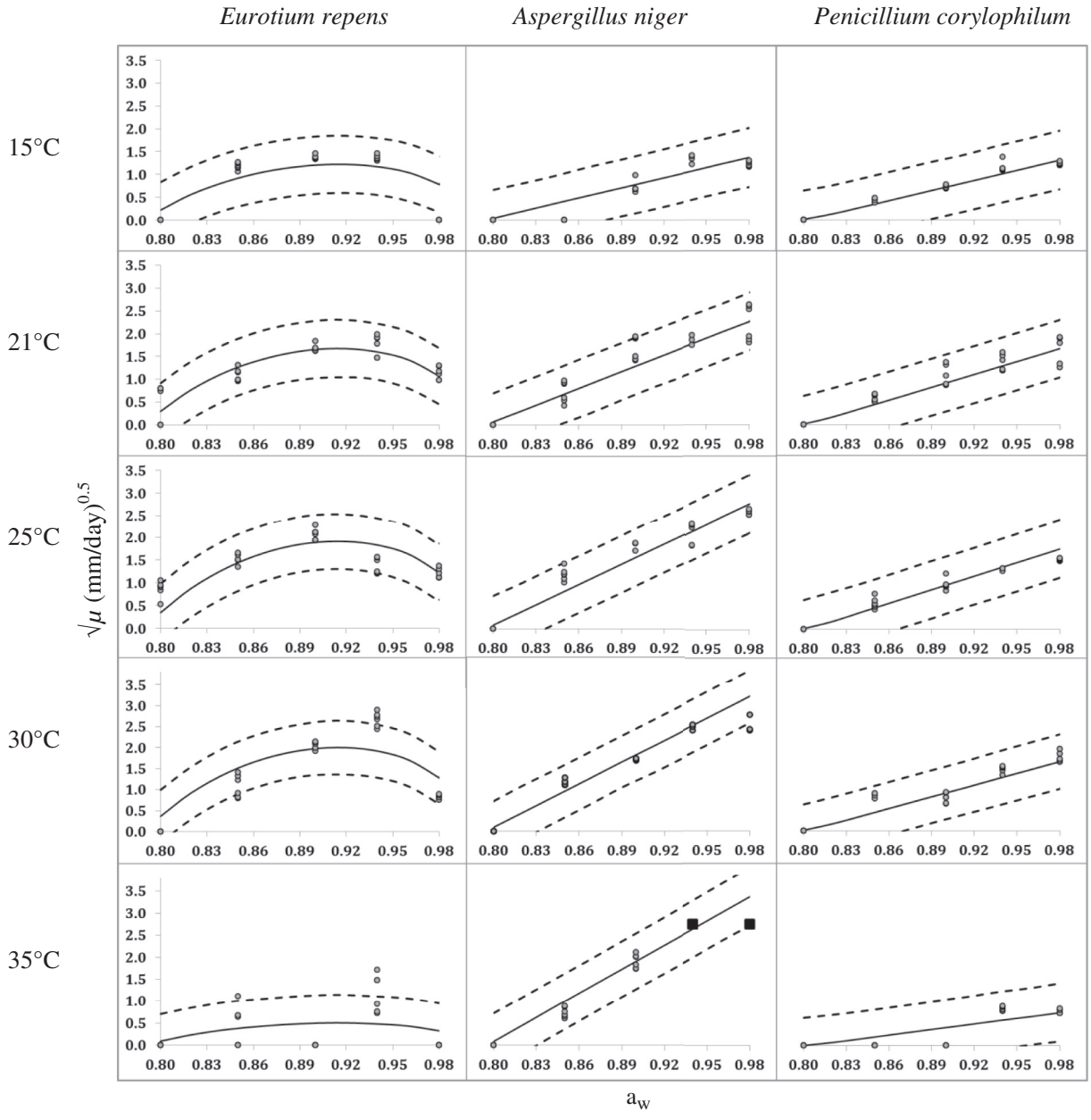

Fig. 1. Fit of the radial growth rate model (Eq. (2)) on the transformed growth rate data $\sqrt{(\mu)}$ versus a_w at five constant temperatures for *Eurotium repens*, *Aspergillus niger* and *Penicillium corylophilum*. Observed growth rate data (○) and right censored data (■); model (solid lines) and 95% credibility interval (dashed lines).

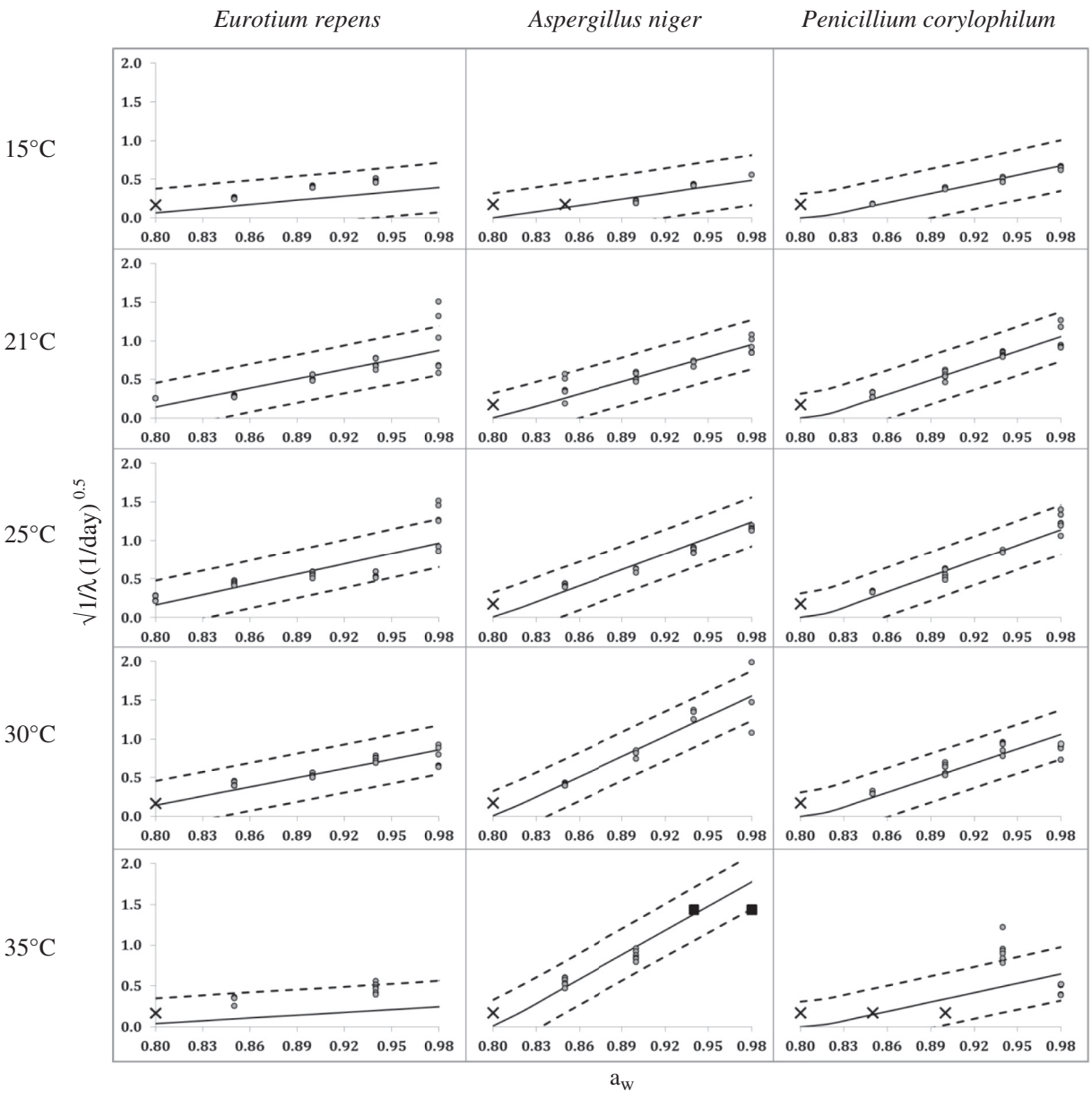

Fig. 2. Fit of the lag time for growth model (Eq. (3)) on the transformed lag time data ($\sqrt{1/\lambda}$) versus a_w at five constant temperatures for *Eurotium repens*, *Aspergillus niger* and *Penicillium corylophilum*. Observed lag time data (○), right censored data (▮) and left censored data (x); model (solid lines) and 95% credibility interval (dashed lines).

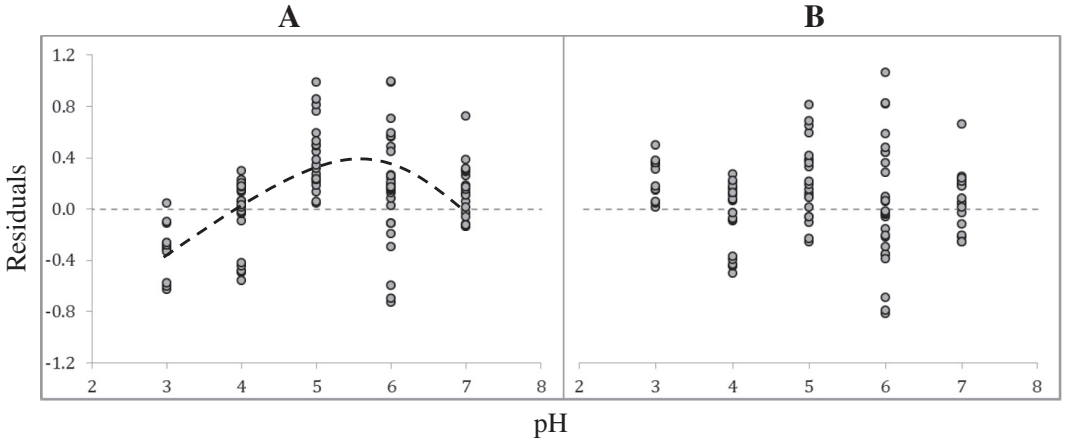

Fig. 3. Residuals (i.e. observed data minus fitted data) versus pH for *Eurotium repens*. (A) radial growth rate model without pH Gamma term (Eq. (2)). (B) Radial growth rate model with pH Gamma term (Eq. (2) completed with Eq. (7)). The black dashed line emphasizes a potential pH effect.

Table 6
Estimated parameters of the radial growth rate model (Eq. (2)) completed with the pH Gamma term (Eq. (7)) applied to *Eurotium repens*.

		Mean	sd	Lower 95% CI	Upper 95% CI
Temperature (°C)	T_{min}	3.5	3.5	−4.4	9.4
	T_{opt}	27.2	1.1	25.2	29.4
	T_{max}	35.3	0.1	35.1	35.6
Water activity	$a_{w\ min}$	0.786	4.33×10^{-3}	0.786	0.793
	$a_{w\ opt}$	0.917	4.16×10^{-3}	0.909	0.925
pH	pH_{min}	2.5	0.2	2.5	2.7
	pH_{opt}	5.6	0.2	5.6	6.2
	pH_{max}	8.8	0.6	8.1	10.3
$\sqrt{\mu^{opt}}$ (mm/day)$^{0.5}$		2.30	0.08	2.14	2.45
σ_μ		0.36	0.02	0.32	0.41

successfully to fungi, for example in Deschuyffeleer et al. (2013), Gougouli et al. (2011) and Rosso and Robinson (2001).

The originality of the study relies on developing Gamma models (i) for both radial growth rate and lag time for growth, and (ii) for three different mold species representative of bakery spoilage. With both radial growth rate and lag time, it is now possible to calculate the consumer rejection time, defined as the sum of the lag time for growth and the time to reach a visible limit when the mycelium growth starts (Horner and Anagnostopoulos, 1973), for any a_w and temperature combination (Eq. (13)). This model development step is a prerequisite toward shelf-life prediction (Dagnas and Membré, 2013; Gougouli et al., 2011).

$$t_K^v = \lambda_K + \frac{D_v}{\mu_K} \tag{13}$$

In Eq. (13) K, μ and λ are the same as in Eqs. (2) and (3), t_v is the time to visible growth (also known as the rejection time) and D_v is the visible diameter and generally set to 3 mm (Gougouli et al., 2011).

Moreover, having predictive models developed for three mold species involved in the spoilage of a given food product enables to have a robust assessment of the microbial shelf-life.

In addition, the development of predictive models for three different mold species offers the opportunity to compare the mold behavior through their model structure. Our results showed that the same model framework (i.e. one model structure whatever the mold species, regarding water activity, temperature and pH) to assess growth was not applicable.

Firstly, it was demonstrated that mycelium of *E. repens* grows faster at low a_w values (c.a. 0.91), while optimum mycelium growth of *A. niger* and *P. corylophilum* occurs at high a_w (>0.98). These two different behaviors led to two different model structures. Secondly, *E. repens* was affected by pH, in the range 3–8, whereas *A. niger* and *P. corylophilum* were not. This finding differs from what is generally reported in the literature, i.e. the factor pH is often considered as having no effect on mold growth within the range 3–8 (Sautour et al., 2001b; Wheeler et al., 1991).

On the other hand, for the temperature effect, the same Gamma term structure (Eqs. (10) and (12)) was applied on the three species, to predict both growth rate and lag time. Likewise, same Gamma terms for a_w and temperature were successfully applied to the three species lag time. Although this result is promising, it has to be interpreted with care. For instance, Gock et al. (2003) have shown that *Xeromyces bisporus* germinated faster (i.e. shorter lag time) at very low a_w (i.e. from 0.86 to 0.89) than at a_w close to 1. In such a case, modeling the effect of a_w on the lag time of *X. bisporus* should lead to a different model structure from what has been developed in this study, for example a cardinal model with $n = 2$ (same structure as Eq. (8)) instead of Eq. (11), might be appropriate. Overall, it seems reasonable to conclude that the behavior of the mold species varies sufficiently for not being described by a unique growth model structure. Consequently, for future research with other mold species, it will be necessary to verify which model structure has to be selected, that is a process which might be time and effort consuming. Nevertheless, our study confirmed that the Gamma model (within a short selection of Gamma terms) described satisfactorily the mold growth behavior as a function of the environmental factors.

Gougouli and Koutsoumanis (2010) were among the first authors that studied the relationship between μ and λ of filamentous fungi. In their study, the product μ times λ was found relatively constant for *A. niger* and *Penicillium expansum* at different storage temperatures and then the authors concluded that a proportional relationship between lag time and growth rate may be verified for mold growth. In their study on *Aspergillus candidus*, Huchet et al. (2013) have quantified the effect of temperature and a_w on the radial growth rate, and assuming μ times λ constant, calculated the lag time for growth before concluding on shelf-life recommendations. In our study, whereas a pattern was noticed between $\sqrt{\mu}$ and $\sqrt{1/\lambda}$, the product μ times λ was not constant enough to conclude to proportionality between these two growth responses for *A. niger* and *P. corylophilum*. Moreover, for *E. repens* the product μ times λ was not constant, and the impacts of a_w on lag time and mycelium growth were significantly different and consequently modeled differently. Overall, the lag time for growth and mycelium growth process correspond to two different, although subsequent, biological phenomena. The lag time studied here encompasses lag time before germination (spore activation), spore swelling, polarized growth (germ tube formation) and exponential hyphal elongation (d'Enfert, 1997; Gougouli and Koutsoumanis, 2013), while mycelium growth corresponds to hyphal division and mycelium elongation. In our opinion, the effect of environmental factors on lag time and mycelium growth rate should be modeled separately.

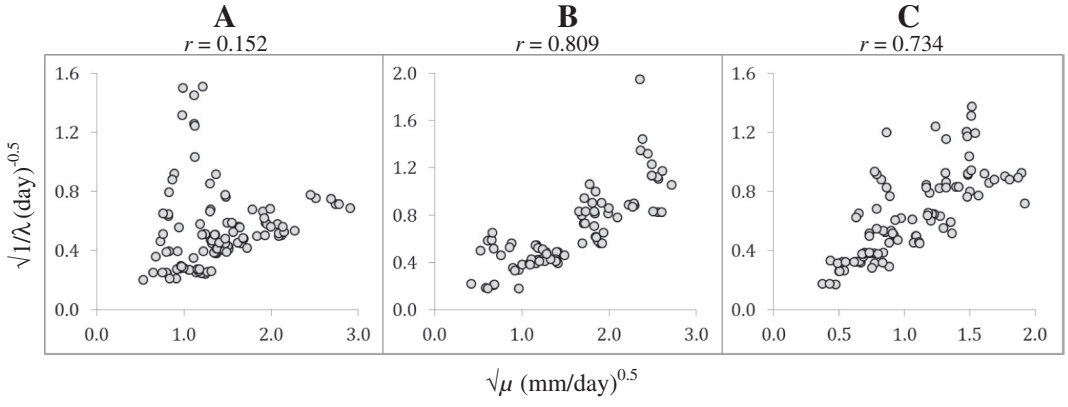

Fig. 4. Relation between inverse of observed lag times for growth ($1/\lambda$) and observed radial growth rates (μ) after square root transformation for *Eurotium repens* (A), *Aspergillus niger* (B) and *Penicillium corylophilum* (C) with their correlation coefficients (r).

Table 7
Minimal, optimal and maximal values of temperature and water activity for which growth and germination kinetics were observed: data reported from literature with their standard deviations (sd) when available. n.a.: not available; λ: lag time for mycelium growth and μ: mycelium radial growth rate.

Species	T_{min} (sd) °C	T_{opt} (sd) °C	T_{max} (sd) °C	$a_{w\ min}$ (sd)	$a_{w\ opt}$ (sd)	a_w depressant	Kinetic response	Comment	Reference
Aspergillus niger	<10	n.a.	n.a.	n.a.	0.98–0.995	Glycerol or glucose	Growth	Derived from observations	(Belfi et al., 2004)
	10.7 (0.8)	34.3 (1.3)	n.a.	n.a.	n.a.	None	μ	Secondary model estimates	(Cuppers et al., 1997)
	10.1 (0.3)	31.4 (0.2)	43.1 (0.1)	n.a.	n.a.	None	μ	Secondary model estimates	(Gougouli and Koutsoumanis, 2010)
	6.8 (0.6)	33.4 (0.3)	47.1 (0.4)	n.a.	n.a.	None	λ	Secondary model estimates	(Gougouli and Koutsoumanis, 2010)
	7.5 (1.4)	33.8 (0.5)	44.3 (0.7)	n.a.	n.a.	None	Germination rate	Secondary model estimates	(Gougouli and Koutsoumanis, 2012)
	4.0 (1.1)	36.7 (0.3)	44.5 (0.6)	n.a.	n.a.	None	Lag time for germination	Secondary model estimates	(Gougouli and Koutsoumanis, 2012)
	9.6 (0.6)	34.0 (0.3)	46.9 (1.0)	n.a.	n.a.	None	μ	Secondary model estimates	(Gougouli et al., 2011)
	6.6 (0.8)	35.8 (0.3)	48.0 (1.6)	n.a.	n.a.	None	λ	Secondary model estimates	(Gougouli et al., 2011)
	6–8	35–37	45–47	n.a.	n.a.	None	Growth	Derived from observations	(Panasenko, 1967)
	n.a.	35	n.a.	n.a.	≥0.98	Glycerol	μ	Derived from observations	(Leong et al., 2006)
	8	n.a.	n.a.	n.a.	n.a.	None	Growth	Derived from observations	(Palacios-Cabrera et al., 2005)
	n.a.	n.a.	n.a.	0.77	n.a.	Agar-cellulose strip	Growth	Derived from observations	(Ayerst, 1969)
Eurotium repens	4–5	25–27	35–37 and 38–40	n.a.	n.a.	None	Growth	Derived from observations	(Panasenko, 1967)
	7	24	38	0.71	0.93	Agar-cellulose strip	Growth	Derived from observations	(Ayerst, 1969)
	n.a.	n.a.	n.a.	0.831	n.a.	NaCl	Germination	Derived from observations	(Andrews and Pitt, 1987)
	n.a.	n.a.	n.a.	0.721	n.a.	Glycerol	Germination	Derived from observations	(Andrews and Pitt, 1987)
	n.a.	n.a.	n.a.	0.685	n.a.	Glucose/fructose	Germination	Derived from observations	(Andrews and Pitt, 1987)
	n.a.	n.a.	n.a.	0.70	n.a.	Glucose/fructose	Germination	Derived from observations	(Gock et al., 2003)
	n.a.	n.a.	n.a.	0.74	n.a.	Glucose/fructose	Growth	Derived from observations	(Gock et al., 2003)
Penicillium corylophilum	n.a.	n.a.	n.a.	≥0.80	n.a.	Glycerol	Growth	Derived from observations	(Abellana et al., 2001)
	n.a.	n.a.	n.a.	<0.85	n.a.	Glycerol	Growth	Derived from observations	(Marín et al., 2002a)
	n.a.	n.a.	n.a.	≥0.80	n.a.	Cake analogue and water	Growth	Derived from observations	(Marín et al., 2002b)
	n.a.	n.a.	37	n.a.	n.a.	None	Growth	Derived from observations	(Pitt and Hocking, 2009)

In conclusion, the effect of a_w, temperature, and pH on growth (lag time for mycelium growth and mycelium radial growth rate) of three molds isolated from spoiled bakery products has been quantitatively assessed. It was demonstrated that the same model structure can be applied to describe the temperature effect on mold growth whatever the species. In contrast, the effect of a_w on *E. repens* mycelium growth differed from the two other species. Further investigation would be necessary to draw a definitive conclusion on the potential relationship between lag time and growth rate; at this stage, it seems cautious to keep modeling these two responses separately when assessing product shelf-life.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ijfoodmicro.2014.06.022.

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