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Modelling growth of *Penicillium expansum* and *Aspergillus niger* at constant and fluctuating temperature conditions

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ABSTRACT

The growth of *Penicillium expansum* and *Aspergillus niger*, isolated from yogurt production environment, was investigated on malt extract agar with pH = 4.2 and $a_w = 0.997$, simulating yogurt, at isothermal conditions ranging from −1.3 to 35 °C and from 5 to 42.3 °C, respectively. The growth rate (μ) and (apparent) lag time (λ) of the mycelium growth were modelled as a function of temperature using a Cardinal Model with Inflection (CMI). The results showed that the CMI can describe successfully the effect of temperature on fungal growth within the entire biokinetic range for both isolates. The estimated values of the CMI for μ were $T_{min} = -5.74$ °C, $T_{max} = 30.97$ °C, $T_{opt} = 22.08$ °C and $\mu_{opt} = 0.221$ mm/h for *P. expansum* and $T_{min} = 10.13$ °C, $T_{max} = 43.13$ °C, $T_{opt} = 31.44$ °C, and $\mu_{opt} = 0.840$ mm/h for *A. niger*. The cardinal values for λ were very close to the respective values for μ indicating similar temperature dependence of the growth rate and the lag time of the mycelium growth. The developed models were further validated under fluctuating temperature conditions using various dynamic temperature scenarios. The time–temperature conditions studied included single temperature shifts before or after the end of the lag time and continuous periodic temperature fluctuations. The prediction of growth at changing temperature was based on the assumption that after a temperature shift the growth rate is adopted instantaneously to the new temperature, while the lag time was predicted using a cumulative lag approach. The results showed that when the temperature shifts occurred before the end of the lag, they did not cause any significant additional lag and the observed total lag was very close to the cumulative lag predicted by the model. In experiments with temperature shifts after the end of the lag time, accurate predictions were obtained when the temperature profile included temperatures which were inside the region of growth, showing that the assumption that μ is adopted instantaneously to the current temperature is concrete. In contrast, for scenarios with temperatures close or outside the growth region the models overestimated growth, indicating that fungi were stressed by this type of temperature shifts. The present study provides useful data for understanding the behavior of *P. expansum* and *A. niger* at dynamic temperature conditions while the developed models can be used as effective tools in assessing the risk of fungal spoilage and predicting shelf life of foods.

1. Introduction

The growth of filamentous fungi in foods is an important quality problem and may lead to significant economic losses for the food industry (Andersen and Thrane, 2006). Among food products, yogurt is susceptible to fungal spoilage (ICMSF, 2000; Montagna et al., 1998). Fungi can grow in moist environments found in dairy plants, and establish themselves on ceilings, floors, walls and even in floor drains if these areas are not properly cleaned and sanitized (Cousin, 2002). Contamination of yogurt during production may occur through air in packaging areas, contaminated packaging material, starter cultures, ingredients, stabilizing agents and poor hygiene on the processing lines (Cousin, 2002; ICMSF, 2000; Ndagijimana et al., 2008; Ottaviani and Ottaviani, 2003). Fungal spore germination and growth on yogurts depend on intrinsic (e.g., extent of initial contamination and type of contaminants, pH, a_w, composition of the product and natural microflora) and extrinsic (e.g., temperature and packaging atmosphere) factors. Among these, temperature and type of contaminants are considered as the most important for yogurt spoilage by fungi (ICMSF, 2000; Muir, 1996; Viljoen et al., 2003). Genera such as *Penicillium* and *Aspergillus* are the most frequent contaminants of yogurts, and their prevalence is critical for the shelf life of these products because they can grow abundantly at the yogurt/air interface if conditions are favorable (Cousin, 2002; Montagna et al., 1998; Muir, 1996; Ndagijimana et al., 2008). Once contamination occurs, the appearance of a visible mycelium on yogurt depends on the spore germination time and the mycelium growth rate which are strongly affected by the temperature conditions during transport, distribution and storage of the product. Considering that the above factors can vary

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significantly, an effective shelf life evaluation requires information on the effect of storage temperature on the mycelium growth kinetics expressed in quantitative terms (i.e. predictive models).

Recent advances in predictive microbiology and mycology have allowed the development of effective validated models pertinent to food safety and quality. The last decade an increased number of predictive models for fungal growth have been developed (Lahlali et al., 2005; Marín et al., 2009; Panagou et al., 2003, 2007; Pardo et al., 2005, 2006; Plaza et al., 2003; Rosso and Robinson, 2001; Samapundo et al., 2005, 2007; Sautour et al., 2001; Tassou et al., 2007). Most of these models focus on the effect of a_w, or the combined effect of temperature and other environmental factors on growth, while the available models on the individual effect of temperature in the whole biokinetic range of fungal growth are very limited. In addition, the existing studies on the effect of temperature on fungal growth have been carried out at isothermal conditions. However, temperature fluctuations are frequently encountered during distribution and storage (Giannakourou et al., 2005; Koutsoumanis, 2001; Laguerre et al., 2002). Although numerous published models have dealt with the prediction of bacterial growth under dynamic temperature conditions (Gougouli et al., 2008; Koutsoumanis, 2001; Koutsoumanis et al., 2006; Mellefont and Ross, 2003; Zwietering et al., 1994), reports on fungal behavior under temperature fluctuations are still missing.

The objectives of the present work were: i) to study and model the effect of temperature on the mycelium growth of *P. expansum* and *A. niger*, ii) to investigate the effect of temperature fluctuations on the growth of the above fungi and iii) to examine the ability of the developed models to predict fungal growth under dynamic temperature conditions.

2. Materials and methods

2.1. Strains and growth media

One *A. niger* strain (code AN-YV7) and one *P. expansum* strain (code PE-YV1), kindly provided by a Greek dairy industry, were used throughout the study. Both strains were isolated from the environment of the yogurt production unit and are deposited in the strain collection of the Laboratory of Food Microbiology and Hygiene of Aristotle University of Thessaloniki. The isolates were maintained on sterile distilled water containing 0.1% wetting agent (Tween 80; Merck, Darmstadt, Germany) at 5 °C. The standard growth medium used in all experiments was malt extract agar (MEA; LAB M Limited, Lancashire, United Kingdom). The medium was adjusted to pH 4.2 by adding lactic acid (Fluka, Buchs, Germany), simulating the pH of yogurt. The pH and a_w of the medium were measured at 25 °C using a pH meter (pH 211 Microprocessor, Hanna Instruments BV, Ijsselstein, the Netherlands) and an Aqualab Series 3 water activity determination device (Decagon Devices Inc., Pullman, WA, United States), respectively. The a_w of the medium was monitored throughout the experiments and no significant changes were observed. Autoclaved medium (20 mL) was poured into 90 mm diameter sterile plastic Petri dishes and used for inoculation immediately.

Table 1
Kinetic parameters of *P. expansum* and *A. niger* stored under different isothermal conditions.

T (°C) [a]	*P. expansum*		T (°C)	*A. niger*	
	μ (mm/h) [b,c]	λ (h) [b,c]		μ (mm/h) [b,c]	λ (h) [b,c]
−1.3	0.008 ± 0.001	709.4 ± 175.6	12.9	0.045 ± 0.003	91.8 ± 4.8
0.5	0.023 ± 0.001	249.6 ± 24.4	15.4	0.115 ± 0.007	51.2 ± 2.9
4.7	0.053 ± 0.001	105.2 ± 0.9	20.2	0.384 ± 0.004	24.3 ± 0.5
11.0	0.124 ± 0.002	48.3 ± 3.1	24.8	0.630 ± 0.005	16.7 ± 0.7
15.4	0.165 ± 0.005	23.5 ± 2.0	30.3	0.856 ± 0.019	13.3 ± 0.8
20.2	0.225 ± 0.002	15.5 ± 2.4	32.8	0.866 ± 0.030	13.4 ± 0.5
24.8	0.232 ± 0.009	14.3 ± 1.0	35.0	0.839 ± 0.030	12.3 ± 0.5
27.3	0.142 ± 0.002	16.2 ± 1.6	37.1	0.670 ± 0.010	14.5 ± 1.1
30.3	0.041 ± 0.005	63.2 ± 8.3	40.0	0.301 ± 0.005	15.2 ± 1.6
			42.3	0.133 ± 0.003	21.6 ± 2.9

[a] Temperature in the agar during storage as recorded by the temperature monitoring devices.
[b] μ, growth rate; λ, lag time.
[c] Mean value (± the standard deviation) of six replicates.

2.2. Inoculation, incubation conditions and growth measurements

A. niger and *P. expansum* were grown on MEA for 5 and 7 days, respectively, at 25 °C to obtain heavily sporulating cultures. Spores were then suspended in sterile distilled water containing 0.1% of Tween 80 by scraping gently the surface of the medium with a sterile spatula. After filtering the spore suspension through four layers of sterile medical tissue (Aseptica, Athens, Greece), its final concentration was assessed using a Neubauer counting chamber (Precicolor, HBG, Germany). Immediately after preparation, a 1-μL aliquot of the suspension (final concentration 10^6 spores/μL) was inoculated in the centre of the Petri dishes containing the solidified growth medium. The inoculated plates were sealed with parafilm and stored under controlled isothermal storage conditions in high-precision (±0.2 °C) low-temperature programmable incubators (model MIR 153, Sanyo Electric Co., Ora-Gun, Gunma, Japan) which were set at −1.5, 0, 5, 10, 15, 20, 25, 27.5, 30, 33

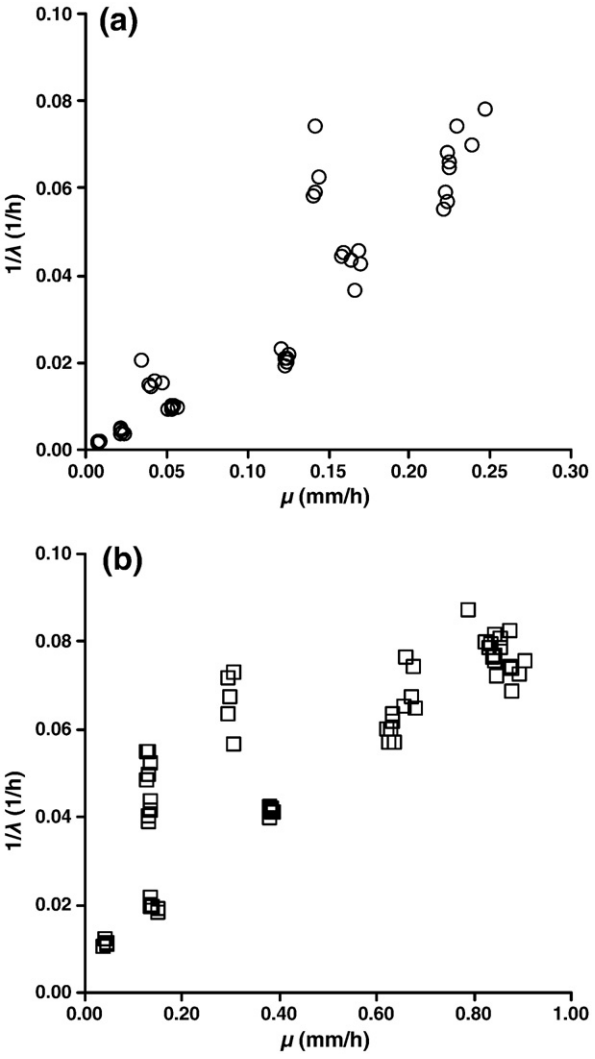

Fig. 1. Relation between observed lag time and mycelium growth rate for *P. expansum* (a) and *A. niger* (b).

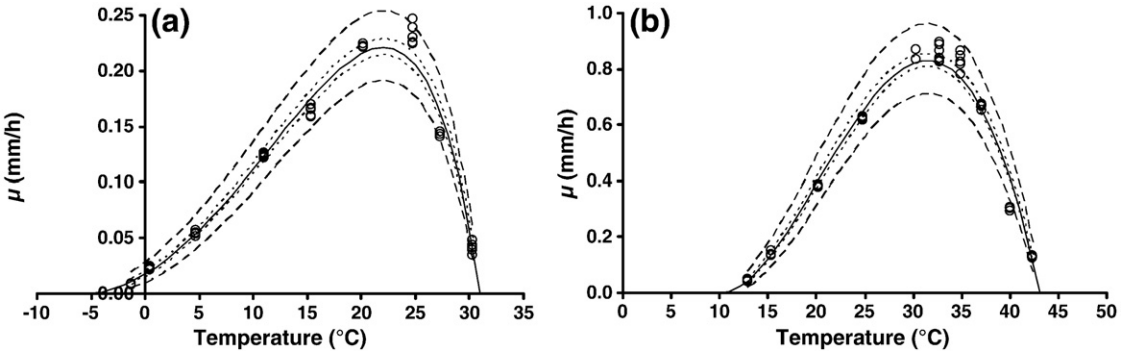

Fig. 2. Effect of storage temperature on the growth rate (μ) of *P. expansum* (a) and *A. niger* (b) fitted with Cardinal Model with Inflection (solid line). Points (○) represent observed values of the growth rate. The dotted and the discontinuous lines depict the 95% confidence and prediction limits of the effect of storage temperature on the growth rate, respectively.

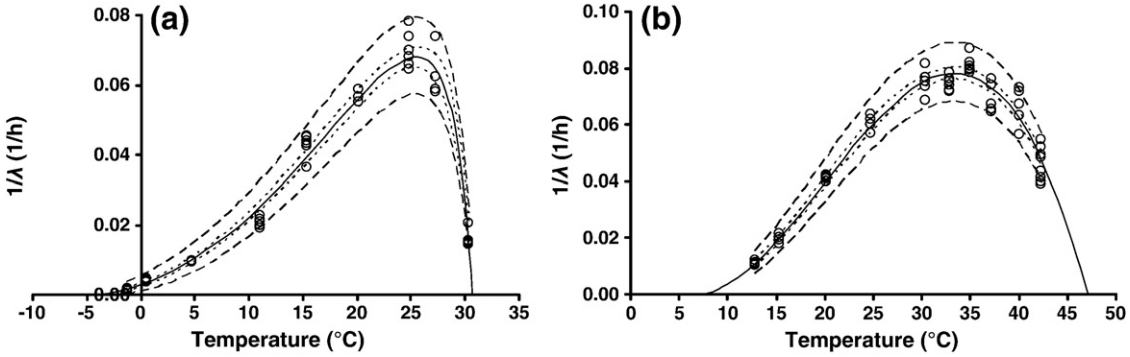

Fig. 3. Effect of storage temperature on the lag time (λ) of *P. expansum* (a) and *A. niger* (b) fitted with Cardinal Model with Inflection (solid line). Points (○) represent observed values of the lag time. The dotted and the discontinuous lines represent the 95% confidence and prediction limits of the effect of storage temperature on the lag time, respectively.

and 35 °C for *P. expansum* and at 5, 10, 12.5, 15, 20, 30, 33, 35, 37, 40 and 42.5 °C for *A. niger*. During storage the inoculated Petri dishes were examined at appropriate time intervals and perpendicular diameters (mm) of the mycelium were measured without opening the dishes using a ruler, to allow for an efficient kinetic analysis. The time of measurement of 1 min did not cause any temperature disturbance, as it was shown from the data loggers. Measurements were carried out for a maximum of 90 days. The growth data observed under isothermal conditions were used to develop the experimental design for monitoring the growth under non-isothermal conditions. The time–temperature scenarios studied included single temperature shifts before or after the end of lag time and continuous periodic temperature fluctuations. For the experiments at dynamic temperature conditions high-precision programmable incubators (model MIR 153, Sanyo Electric Co., Ora-Gun, Gunma, Japan) were used. The temperature during the experiments was monitored using electronic data loggers (Cox Tracer, Cox Technologies, Belmont N.C., United States) with the internal and external sensors monitoring the temperature of the incubator and agar, respectively. No significant difference was observed between the incubator's temperature and the medium's temperature for all profiles tested. For each strain and each storage temperature two individual experiments with three replicated plates for each experiment were tested ($n=6$).

2.3. Modelling growth at isothermal conditions

The diameter of the colony was plotted against time and fitted to a two-phase linear model for the estimation of the growth rate (mm/h) and the lag time (h) using Microsoft Excel.

$$D_{(t)} = \begin{cases} D_0 & \text{if } t \le \lambda \\ D_0 + \mu(t-\lambda) & \text{if } t > \lambda \end{cases} \quad (1)$$

where t is time (h), $D_{(t)}$ is the diameter at time t and D_0 is the diameter of the inoculated spore suspension drop ($D_o = 4$ mm).

Table 2
Estimated values and statistics for the parameters of CMI describing the effect of temperature on growth rate (μ) of *P. expansum* and *A. niger*.

Fungus	Parameter	Estimated value	SE[a]	R^2
P. expansum	T_{min} (°C)	−5.74	0.38	0.985
	T_{max} (°C)	30.97	0.07	
	T_{opt} (°C)	22.08	0.23	
	μ_{opt} (mm/h)	0.221	0.004	
A. niger	T_{min} (°C)	10.13	0.27	0.984
	T_{max} (°C)	43.13	0.07	
	T_{opt} (°C)	31.44	0.22	
	μ_{opt} (mm/h)	0.840	0.012	

[a] Standard error.

Table 3
Estimated values and statistics for the parameters of CMI describing the effect of temperature on reverse lag time (1/λ) of *P. expansum* and *A. niger*.

Fungus	Parameter	Estimated value	SE[a]	R^2
P. expansum	T_{min} (°C)	−5.21	0.44	0.984
	T_{max} (°C)	30.68	0.06	
	T_{opt} (°C)	25.46	0.31	
	$1/\lambda_{opt}$ (1/h)	0.068	0.001	
A. niger	T_{min} (°C)	6.67	0.57	0.976
	T_{max} (°C)	47.10	0.44	
	T_{opt} (°C)	33.38	0.28	
	$1/\lambda_{opt}$ (1/h)	0.078	0.001	

[a] Standard error.

The estimated μ and λ for the two strains at isothermal conditions were further expressed as a function of temperature using the Cardinal Model with Inflection (CMI) originally developed by Rosso et al. (1993):

$$\mu = \frac{\mu_{opt}(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]} \quad (2)$$

$$1/\lambda = \frac{(1/\lambda_{opt})(T-T_{max})(T-T_{min})^2}{(T_{opt}-T_{min})[(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)]} \quad (3)$$

where T_{opt}, T_{min} and T_{max} are the theoretical optimum, minimum and maximum temperature for growth (°C), respectively, and μ_{opt} and λ_{opt} are the growth rate and the lag time at the optimum temperature,

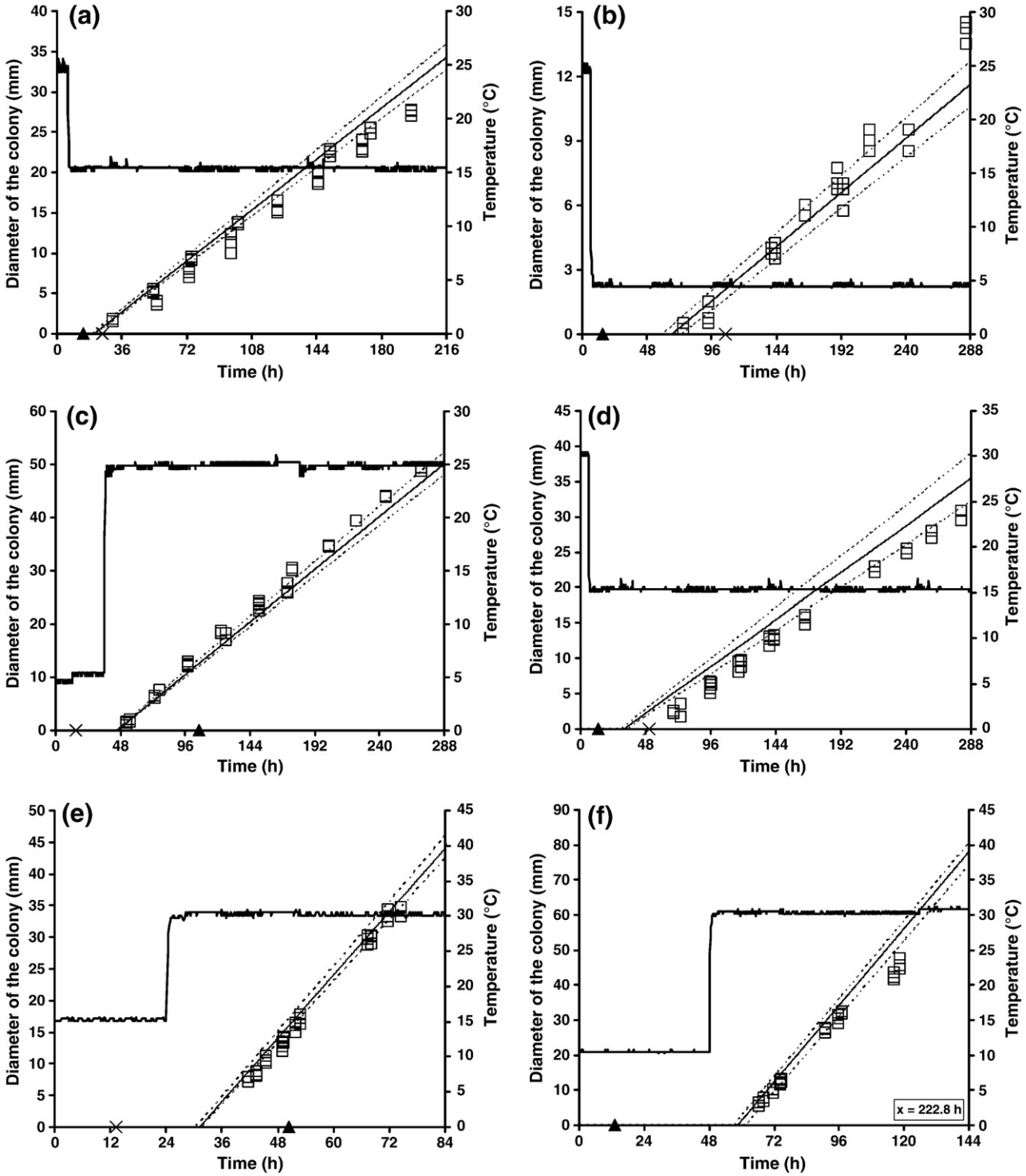

Fig. 4. Comparison between observed (points) and predicted (lines) growth of *P. expansum* under dynamic temperature conditions with temperature shift occurring before the end of the lag (a: 6 h at 25 °C and then at 15 °C; b: 6 h at 25 °C and then at 5 °C; c: 36 h at 5 °C and then at 25 °C) and *A. niger* (d: 6 h at 30 °C and then at 15 °C; e: 24 h at 15 °C and then at 30 °C; f: 48 h at 10 °C and then at 30 °C). The points on the *x*-axis show the predicted lag time at the temperature before (▲) and after (*x*) the temperature shift. The dotted lines depict the 95% confidence limits of predicted growth.

respectively. In order to stabilise the variance a square root transformation was used. The Table Curve 2D software (Systat Software Inc., San Jose, CA, United States) was used for fitting the data to the models.

2.4. Validation of models at dynamic temperature conditions

The developed models were further validated against observed fungal growth at fluctuating temperature conditions. The prediction of growth at such conditions was based on the assumption that after a temperature shift, the growth rate is adopted instantaneously to the new temperature environment. For the prediction of the lag time, a cumulative lag approach (Koutsoumanis, 2001) was applied (Eq. (4)). Based on the above, growth under non-isothermal temperature (Eq. (5)) was predicted from Eq. (1) using the tested time–temperature conditions, with $T(t)$ corresponding to the temperature profile recorded by the data loggers (with a time interval of 0.17 h), in conjunction with the CMsI (Eqs. (2) and (3)) for the estimation of the "momentary" $\lambda_{(T)}$ and $\mu_{(T)}$.

$$\int_0^{\lambda} \frac{1}{\lambda_{(T)}} dt = 1 \tag{4}$$

$$D_{(t)} = \begin{cases} D_0 & \text{if } t \le \lambda \\ D_{(t-1)} + \mu_{(T)} dt & \text{if } t > \lambda \end{cases} \tag{5}$$

where λ is the total lag time, $\lambda_{(T)}$ and $\mu_{(T)}$ are the lag time and the growth rate, respectively, corresponding to the temperature T of an assuming constant-temperature short time interval dt and D_t is the diameter of the tested fungus at time t. The values of $\mu_{(T)}$ and $\lambda_{(T)}$ for each temperature are estimated from Eqs. (2) and (3). The 95% confidence intervals of the predicted growth were estimated based on the confidence interval of the CMI.

3. Results and discussion

The data on the mycelium growth of both *P. expansum* and *A. niger* in MEA at pH = 4.2 and a_w = 0.997 showed that, after an initial lag period, the increase in mycelium diameter over time was a straight line for all storage temperatures tested. The growth rate was determined with a good accuracy by using the linear model as described in Eq. (1). Regression coefficients were in the range of 0.991–1 and 0.971–1 for *P. expansum* and *A. niger*, respectively. The above linearity of fungal growth has been reported in several studies with various fungi (Baert et al., 2007b; Bellí et al., 2004; Cuppers et al., 1997; Lahlali et al., 2005, 2006; Leong et al., 2006; Nevarez et al., 2009; Pardo et al., 2005; Parra and Magan, 2004; Sautour et al., 2001).

The estimated μ and λ values for the two tested fungi at the different storage temperatures are presented in Table 1. For *P. expansum*, the lower storage temperature at which growth was observed was −1.3 °C. At this temperature a very slow increase of the mycelium diameter was observed after an extensive lag period of about one month. The fastest growth was observed at 20 and 25 °C, while the maximum temperature at which growth occurred was 30.3 °C. At the latter temperature, the mycelium had a different morphology (white without spore formation), as it has been previously reported by Baert et al. (2007a). The above results are in agreement with the findings of other published studies on the growth of *P. expansum* (Baert et al., 2007a,b; Lahlali et al., 2005; Pitt and Hocking, 1997a) or other Penicillia such as *P. glabrum* (Mitchell et al., 2004), *P. chrysogenum* (González et al., 1988), *P. digitatum* and *P. italicum* (Plaza et al., 2003).

The growth of *A. niger* was significantly faster and with shorter lag times compared with the growth of *P. expansum* at all temperatures tested (Table 1). No mycelium growth was observed for *A. niger* at 10 °C in the time frame of the experiments, while the lower temperature at which the fungus managed to grow was 12.5 °C. This temperature is higher compared to the minimum temperatures for growth of 6 and 10 °C reported by Bellí et al. (2004) and Pitt and Hocking (1997b). Faster mycelium growth and shorter lag time were observed at 32.8 °C. Nevertheless, there is evidence suggesting optimum temperature for *A. niger* growth at 30 °C (Vats and Banerjee, 2002), 33 °C (Parra and Magan, 2004), 35 °C (Leong et al., 2006), 37 °C (Marín et al., 1998), 35–37 °C (Pitt and Hocking, 1997b) and 30–37 °C (Bellí et al., 2004). The maximum temperature at which growth of *A. niger* was observed was 42.3 °C, which is slightly lower compared to the findings of other studies, reporting a maximum temperature range for growth between 45 and 47 °C (Pitt and Hocking, 1997b; Vats and Banerjee, 2002).

For both fungi tested, the variation among replicates for μ was significantly lower than that of λ (Table 1). In addition, for both μ and λ the variation between replicates was low at optimum conditions but increased significantly at conditions close to the boundary of growth. For example, for the *P. expansum* lag time, the percent coefficient of variation (%CV = st. dev/mean*100) increased from 7.0% at 24.8 °C to 24.7% at −1.3 °C. The increased variance in fungal growth kinetic parameters at stressful conditions has been also reported by Baert et al. (2008).

In bacterial growth, lag time λ can be determined as the ratio between the amount of "work" that a cell has to perform in order to adapt to its new environment and the rate at which it is able to perform that work which may be identified with maximum specific growth rate μ (Delignette-Muller, 1998; Pirt, 1975; Robinson et al., 1998). In that case, the "work" for adaptation is defined by the product of μ and λ ($\mu^*\lambda$) which is also called "physiological state" of the cells (Baranyi and Roberts, 1994). Several studies have reported a relation between μ and λ with their product $\mu^*\lambda$ being constant at different storage temperatures when the pre-inoculation history of the culture is identical (Baranyi and Roberts 1994; Baranyi et al., 1995; Gougouli et al., 2008; Koutsoumanis et al., 2006; Pin et al., 2002). In contrast to bacterial growth, the relation between μ and λ in fungal growth has not been investigated in detail. In the present study the product $\mu^*\lambda$ was found to be relatively constant for all temperatures and both fungi tested (Fig. 1). This indicates that the mycelium growth rate may be related to the rate at which the "work" required for spore germination is performed. It needs to be noted however, that the estimated lag time of fungal growth is a graphical interpretation of the linear mycelium growth and its relation to the germination time of the spores is not clear. Although studies have shown that for a large number of inoculated spores the lag time of mycelium growth is equal to the germination time (Dantigny et al., 2002, 2006), further research at a single spore level is needed for a better understanding of the relation between mycelium growth kinetics (μ and λ) and germination time.

The estimated kinetic parameters (μ and λ) of growth of the two fungi were further modelled as a function of temperature using the CMI proposed by Rosso et al. (1993). This model has been successfully used for modelling the effect of various environmental factors on fungal growth (Cuppers et al., 1997; Marín et al., 2009; Membré et al., 2001; Panagou

Table 4
Observed and predicted values for the lag time and the growth rate of *P. expansum* and *A. niger* stored under dynamic temperature conditions, with the temperature shift occurring before the end of the lag.

Fungus	Figure	Lag time (h)		Growth rate (mm/h)	
		Observed[a]	Predicted[b]	Observed[a]	Predicted[b]
Penicillium expansum	4a	25.6 ± 5.8	20.7	0.167 ± 0.003	0.175
	4b	80.6 ± 9.6	66.3	0.064 ± 0.009	0.052
	4c	43.2 ± 1.5	45.3	0.212 ± 0.005	0.206
Aspergillus niger	4d	50.7 ± 7.0	32.2	0.137 ± 0.012	0.138
	4e	33.6 ± 0.7	31.2	0.863 ± 0.028	0.838
	4f	58.1 ± 0.7	58.5	0.763 ± 0.030	0.913

[a] Values are means (± the standard deviation).
[b] Predicted values from CMI for the temperature recorded by the temperature monitoring devices.

et al., 2003; Rosso and Robinson, 2001; Sautour et al., 2001; Tassou et al., 2007). The main advantage of this model is that all its parameters can be considered to be biologically interpretable (Cuppers et al., 1997; Ratkowsky, 2004). Such a model can provide estimations of the minimum and maximum values of the environmental factors allowing growth (in this case T_{min} and T_{max}) which are not easy to be determined experimentally because fungal growth can well occur after several months of incubation (Dantigny, 2004). In general however, the values of these cardinal parameters should be considered as theoretical and not interpreted as "true" minimum and maximum temperatures for growth (Ratkowsky, 2004).

The fitting of the models to the observed μ and $1/\lambda$ of *P. expansum* and *A. niger* together with the 95% confidence and prediction limits are presented in Figs. 2 and 3. As it is shown in the latter figures the

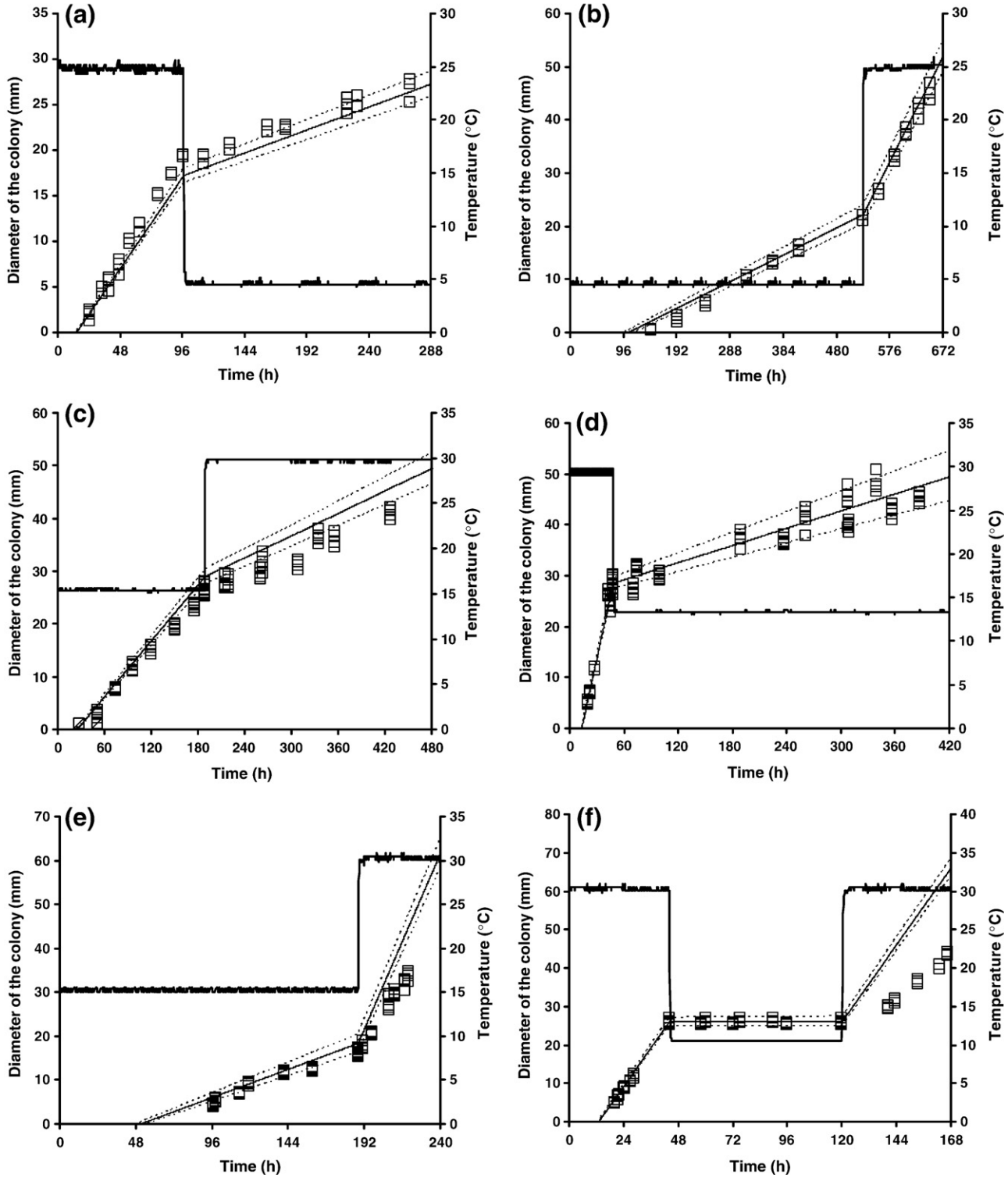

Fig. 5. Comparison between observed (points) and predicted (lines) growth of *P. expansum* under dynamic temperature conditions, with temperature shifts occurring after the end of the lag (a: 97 h at 25 °C and then 191 h at 5 °C; b: 528.5 h at 5 °C and then 143.5 h at 25 °C; c: 189 h at 15 °C, 241 h at 30 °C) and *A. niger* (d: 48 h at 30 °C and then 372 h at 13 °C; e: 188 h at 15 °C and then 52 h at 30 °C; f: 48 h at 30 °C, 72 h at 10 °C and then 48 h at 30 °C). The dotted lines depict the 95% confidence limits of predicted growth.

model fitted well the observed data for both fungi tested. The estimated cardinal values are presented in Tables 2 and 3. In general, the CM values were close to the minimum, maximum and optimum temperatures for growth observed experimentally. In addition, the estimated values of the CMI for $1/\lambda$ were close to the respective values for μ indicating similar temperature dependence for the growth rate and the lag time of the mycelium growth.

Studies on the chill chain have shown that significant temperature fluctuations may occur during transport, retail or domestic storage of foods (Giannakourou et al., 2005; Koutsoumanis, 2001; Laguerre et al., 2002). Thus, effective predictions of fungal spoilage require a dynamic model able to predict growth at non-isothermal conditions. The existing mathematical models predicting fungal growth however, refer to constant temperature (Baert et al., 2007b; Nevarez et al., 2009; Samapundo et al., 2007). In addition, the available data on the effect of temperature shifts on mycelium growth are very limited (Gocheva et al., 2009; Xu, 1999). In the present study, the developed models for the effect of temperature on the growth of. *P. expansum* and *A. niger* were further validated at dynamic temperature conditions. Mycelium growth was monitored at various time–temperature scenarios including single temperature shifts before or after the end of lag time and continuous periodic temperature fluctuations, and was compared with model predictions. The prediction of growth at fluctuating temperature (Eq. (5)) was based on the division of the time–temperature history into short, assuming constant-temperature time intervals and the assumption that, after a temperature shift, the growth rate is adopted instantaneously to the new temperature environment (Fu and Labuza, 1993; Koutsoumanis, 2001). For the prediction of the lag time a cumulative lag approach (Fu and Labuza, 1993; Koutsoumanis, 2001) was applied (Eq. (4)). A comparison between observed and predicted growth of *P. expansum* and *A. niger* at scenarios with a single temperature shift before the end of the lag is shown in Fig. 4. The observed and predicted values of μ and λ are presented in Table 4. For all temperature scenarios and for both fungi tested the observed growth was close to the predicted one. Even for an abrupt temperature downshift of 20 °C (from 25 to 5 °C) the model predicted a lag time of 66.3 h with the observed lag being 80.6 h (Fig. 4b). Accurate predictions were also obtained in the case where the first temperature step was 10 °C which is below the minimum temperature for *A. niger* growth (Fig. 4f). Indeed, in the latter scenario the observed lag time was very close to sum of the first time step at 10 °C and the total lag corresponded to the temperature of the second step, indicating that during storage at a temperature below the minimum temperature for growth no lag is consumed. This observation is in agreement with other studies reporting the ability of spores to develop and form a mycelium, after periods of extreme-no growth environments when conditions become favorable again (Beyer et al., 2004; Ottaviani and Ottaviani, 2003). On the whole, the above results show that temperature shifts before the appearance of a visible mycelium do not cause any significant additional lag in mycelium growth and thus, the total lag can be predicted using the cumulative approach described with Eq. (4). However, for a better understanding of fungal behavior at changing environments further research on the effect of temperature shifts on spore germination is required. This will lead to more accurate predictions of fungal growth lag time which are of great importance for avoiding mycelium appearance and subsequently spoilage of foods (Garcia et al., 2009).

The comparison between observed and predicted growth of *P. expansum* and *A. niger* at scenarios with temperature shifts after the end of lag time are shown in Fig. 5. At all tested conditions, with temperature shifts within the growth region and away of the growth boundary, a satisfactory agreement between predicted and observed growth for the profiles presented in Fig. 5a, b, d and e was obtained, and the observed diameters were in general found to be within the confidence limits of the prediction lines. In the temperature scenario of Fig. 5c, a temperature upshift to 30 °C, close to the maximum temperature for *P. expansum* growth ($T_{max} = 30.97$), resulted in a slight overprediction. A more pronounced prediction error was observed in the scenario of Fig. 5f, where the temperature profile included a downshift to 10 °C which is below the minimum temperature for growth of *A. niger* ($T_{min} = 10.13$ °C). In the latter profile, the exposure of *A. niger* at 10 °C for 72 h resulted in an additional lag time and a much

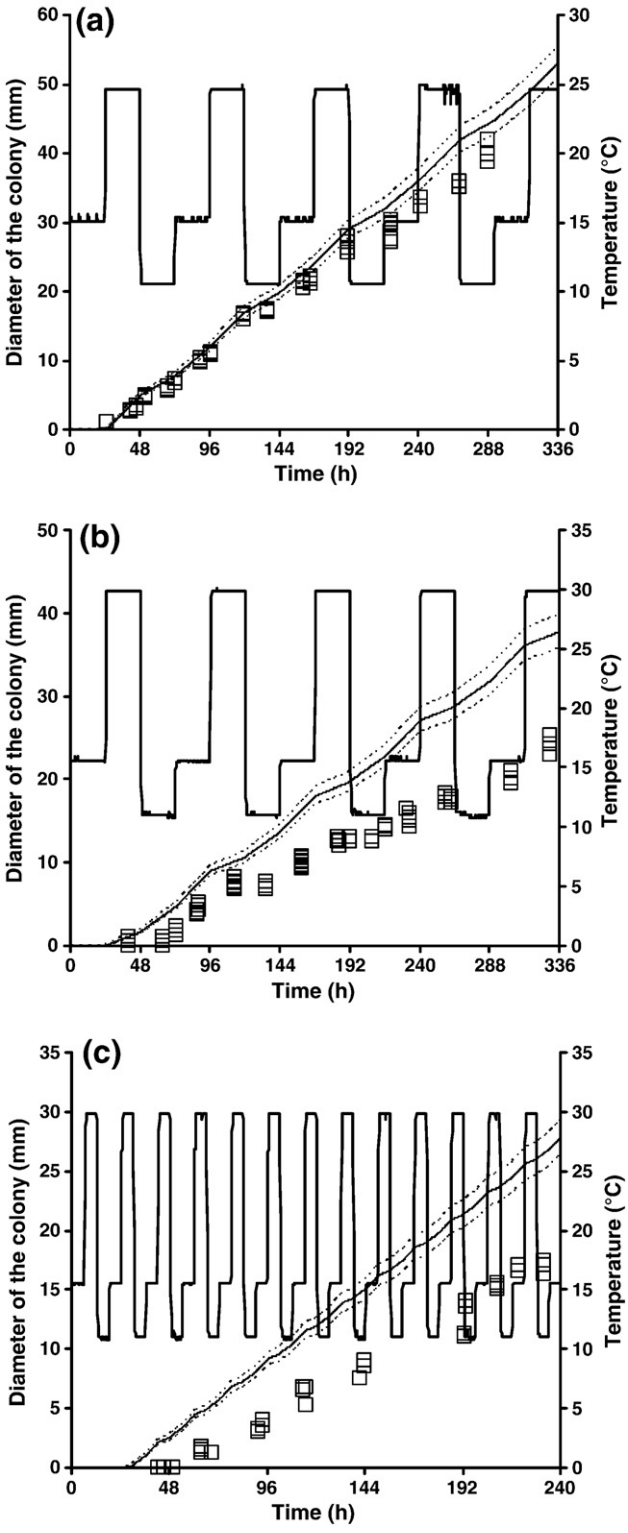

Fig. 6. Comparison between observed (points) and predicted (lines) growth of *P. expansum* stored under periodically temperature fluctuations (a: 24 h at 15 °C, 24 h at 25 °C and 24 h at 10 °C; b: 24 h at 15 °C, 24 h at 30 °C and 24 h at 10 °C; c: 6 h at 15 °C, 6 h at 30 °C and 6 h at 10 °C). The dotted lines depict the 95% confidence limits of predicted growth.

slower growth rate when the storage temperature increased again at 30 °C. The overprediction observed at profiles with temperature shifts close or outside the growth limits were confirmed in the experiments with continuous periodic temperature fluctuations (Figs. 6 and 7). Indeed, in these experiments the models provided satisfactory predictions for profiles with temperatures within the growth region (Figs. 6a and 7a, b), while in scenarios with temperatures close to the growth limits or outside the growth region significant overpredictions of growth were obtained (Fig. 6b, c and 7c). These prediction errors indicate a potential stress imposed by the temperature shifts to low or high temperature. Indeed, several studies have reported that temperature fluctuations and especially cold-shock conditions may induce a stress during fungal growth. For example, Gocheva et al. (2009) demonstrated that a temperature downshift induces an oxidative stress in three strains of the genus *Penicillium* expressed with enhanced levels of oxidative damaged proteins, accumulation of reserve carbohydrates and increased activity of antioxidant enzyme defence. Kawano and Said (2002) reported that *Neurospora crassa* exposed to cold-shock lost its polarized growth, while morphological alteration including dichotomy and branching were detected.

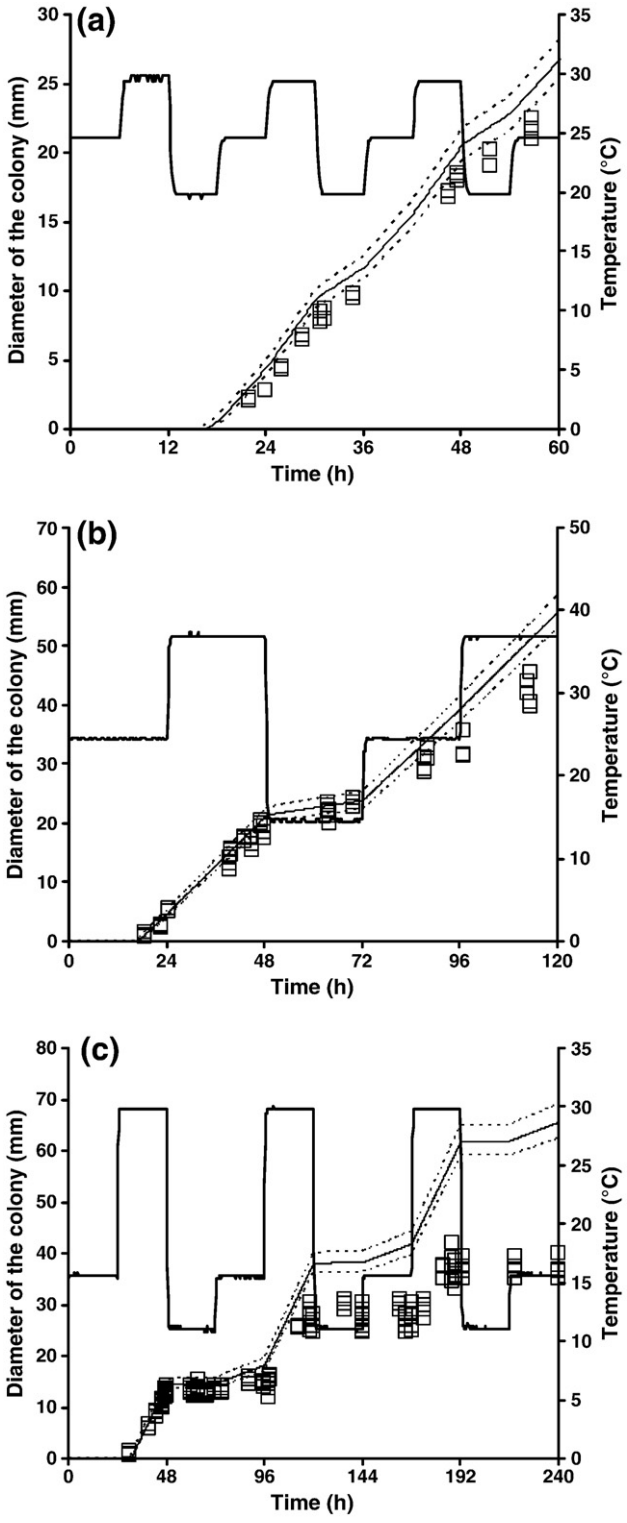

Fig. 7. Comparison between observed (points) and predicted (lines) growth of *A. niger* stored under periodically temperature fluctuations (a: 6 h at 25 °C, 6 h at 30 °C and 6 h at 20 °C; b: 24 h at 25 °C, 24 h at 37 °C and 24 h at 15 °C; c: 24 h at 15 °C, 24 h at 30 °C and 24 h at 10 °C). The dotted lines depict the 95% confidence limits of predicted growth.

In conclusion, the CMI used in the present study described satisfactorily the effect of storage temperature on the mycelium growth of *P. expansum* and *A. niger*. The models provided accurate predictions at dynamic temperature conditions when temperature shifts were within the growth region. On the contrary, increased prediction errors were obtained for storage conditions with temperatures shifts close or outside the growth regions. In these cases however, predictions were in the fail-safe side. With the appropriate validation studies these models can be used as effective tools in shelf life predictions of yogurt or other foods, in the assessment of fungal spoilage risk or the development of decision systems for the optimization of food quality (Giannakourou et al., 2005; Koutsoumanis et al., 2005, 2006).

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