

# Kinetic modelling of *Mucor circinelloides* growth on skim milk agar under dairy-relevant temperature and water activity conditions

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## Abstract

This study investigated the surface growth kinetics of *Mucor circinelloides* strain 1L cultivated on skim milk agar (SMA) under various temperature and water activity ( $a_w$ ) conditions, with relevance to dairy product spoilage prediction. Growth curves fitted using the Baranyi model exhibited high smoothness and reproducibility ( $R^2 > 0.978$ ; RMSE =  $2.36 \pm 1.08$  mm). Primary growth parameters, including lag phase duration and surface growth rate ( $sgr$ ), were quantified across triplicate experiments and modelled using Ratkowsky and cardinal frameworks, respectively. Secondary modelling revealed environmental dependencies, with optimal  $sgr_{opt}$  at 33 mm/d and estimated cardinal parameters ( $T_{opt} \approx 32$  °C, theoretical  $a_{w,min} = 0.97$ ). Validation experiments simulating ewe lump cheese fermentation conditions demonstrated high predictive accuracy ( $R^2 = 0.989$ ), with bias and accuracy factors confirming model reliability despite minor underestimations. Predicted visibility times ( $t_3$ , colony diameter  $\geq 3$  mm) indicated rapid colonisation under favourable conditions, while combinations of reduced temperature ( $< 10$  °C) and low  $a_w$  ( $< 0.975$ ) markedly extended  $t_3$  beyond 30 days. These findings emphasise the value of kinetic modelling for assessing fungal spoilage risk and underpin the need for stringent hygienic measures in dairy production systems.

**Keywords:** surface growth modelling; cardinal model; *Mucor circinelloides*; fungal spoilage

## Introduction

Consumer confidence in the food industry is significantly affected by widespread cases of microbial-induced quality degradation. Microbial spoilage results in considerable economic losses and may also pose a threat to human health. In the food industry, fungal spoilage is a significant concern due to the ability of fungi to thrive under various environmental conditions. Dairy products, particularly cheese, with their rich nutrient content and moisture, provide an ideal substrate for mould growth (Pitt and Hocking, 2022; Shi and Knöchel, 2021a; Ritschard and Schuppler, 2024). Fungal contaminants such as *Mucor* spp. are known for their rapid growth and significant food spoilage. They prefer slightly acidic to neutral pH environments, which are typical of many dairy products. This fungus can contaminate cheese at multiple stages of production, including during milk collection, processing, maturation, and storage (Botha and Botes, 2014; Kure and Skaar, 2019; Garnier et al., 2017a; Garnier et al., 2017b; Biango-Daniels and Wolfe, 2021; Shi and Maktabdar, 2022). *M. circinelloides* can rapidly colonise the cheese surface along with various defects, including visible growth, alterations in colour and/or texture, and the formation of off-odours (Shi and Knöchel, 2021; Morin-Sardin et al., 2016). The most frequent *Mucor* contamination on cheese surfaces is the formation of fluffy white tufts, commonly referred to as the “cat hair” defect. This sudden and invasive phenomenon typically begins within two to five days after cheese production, with the appearance of blackish clumps of mould (Bekada et al., 2008; Morin-Sardin et al., 2017; Kure and Skaar, 2019; Biango-Daniels and Wolfe, 2021).

Numerous reports have linked *Mucor* spp. outbreaks to dairy products (Foschino et al., 1993; Taniwaki et al., 2001; Aboltins et al., 2006) and particularly those involving the *M. circinelloides* species (Lazar et al., 2014; Lee et al., 2014; Snyder et al., 2016; Buehler et al., 2017; Garnier et al., 2017b). These studies underscore the critical importance of addressing fungal spoilage and demonstrate the widespread impact of *M. circinelloides* on cheese production across various scales and regions.

By understanding the growth of fungi associated with food spoilage and implementing effective control measures, the dairy industry can ensure product quality, maintain consumer trust, and comply with safety regulations. Microbiological predictive models are powerful tools that are increasingly involved in quantitative assessment of microbial risk. As an ideal tool for the dairy industry, predictive microbiology provides data for the prediction of shelf life and food quality and safety by combining microbial growth, mathematical models, and statistical data to observe the dynamics of microbial growth in food (Chou et al., 2024; Haque et al., 2024). Dynamic models, in contrast to static predictive models, provide a more comprehensive approach to understanding the complex interactions between fungi, the food matrix, and the surrounding environment in which dairy products are stored or processed. These models allow for real-time prediction of fungal growth in response to intrinsic factors such as pH and water activity ( $a_w$ ), as well as extrinsic factors such as temperature and relative humidity. By estimating various

parameters associated with fungal-induced spoilage, dynamic models facilitate the implementation of more effective control measures in food safety management (Baranyi and Roberts, 1994; Velugoti et al., 2011; Haque et al., 2023; Haque et al., 2024).

To develop effective control measures for mitigating the quality and safety problems of *M. circinelloides* contamination, it is essential to understand fungal growth under static and dynamic temperature conditions. Validation studies can then provide insight into the models' accuracy under realistic scenarios, which can help in day-to-day decision-making in dairy processing operations (Velugoti et al., 2011). Therefore, the objectives of this study were to (i) determine the effect of temperature and  $a_w$  on the mycelial growth of the dairy-associated fungal contaminant *M. circinelloides*, (ii) estimate cardinal values for this dairy isolate, and (iii) to validate *M. circinelloides* mycelial growth with dynamic changes in temperature conditions / under isothermal conditions.

## Materials and methods

### Fungal isolate

The fungal isolate *M. circinelloides* 1L was used for all experiments in the present study. The monitored isolate, which was selected from the collection of the Institute of Food Science and Nutrition (Slovak University of Technology in Bratislava, Slovak Republic), originated from the artisanal Slovak “Bryndza” cheese (Turčianske Teplice, Slovak Republic). Molecular identification was performed based on internal transcribed spacer (ITS) sequencing carried out according to Pangallo et al. (2014). The confirmation was based on the morphological and physiological characteristics following Pitt and Hocking (2022), Botha and Botes (2014) and Samson et al. (2004).

### Experimental design

The levels of the factors were chosen to simulate conditions during the manufacturing, ripening and storage of cheeses and to fully cover the growth region of the species to the greatest possible extent. Growth trials with *M. circinelloides* 1L strain were assessed for fungal growth diameters periodically (10 experimental temperatures  $\times$  3  $a_w$  levels  $\times$  3 growth replicates = 90 growth assays).

### Growth medium

Mycelial growth assessments were performed on Plate Count Skim Milk agar (SMA; Merck, Darmstadt, Germany). Three different  $a_w$  scenarios were implemented: 0.992 $\pm$ 0.002 (unmodified growth medium), 0.985 $\pm$ 0.002 (1 % NaCl, w/v), and 0.975 $\pm$ 0.002 (2 % NaCl, w/v). After sterilisation, 50 mL of medium was poured into sterile Petri dishes (diameter 130 mm)

and  $a_w$  levels were measured with Novasina LabMaster-aw (Novasina, Lachen, Switzerland).

## Inoculation and incubation

The strain was stored on an SMA slant under refrigeration ( $5 \pm 0.5$  °C) to slow down metabolic activity and prevent excessive growth or sporulation during long-term maintenance. To preserve viability, the culture was sub-cultured monthly. For inoculum preparation, a 5-day culture grown on the surface of a Sabouraud slant (Sabouraud Dextrose agar, Biolife Italiana Srl, Milan, Italy) in the dark at  $25 \pm 0.5$  °C was used to promote active growth and abundant sporulation under optimal conditions, resulting in a heavily sporulating culture. For spore collection, the cultures were flooded with sterile saline solution (8.5 g/L NaCl, 0.1 g peptone) containing Tween 80 as a wetting agent (0.01 % v/v). Immediately after preparation, this spore suspension was diluted down and adjusted to  $10^3$  spores/mL and used for inoculation.

Petri dishes were single-point inoculated (2  $\mu$ L) with the fresh suspension to form a circular inoculum in the centre of each SMA plate. Petri dishes with the same water activity level were enclosed in sealed polyethylene bags to avoid  $a_w$  fluctuations, followed by temperature-controlled incubation (Pol-Eko Aparatura, Wodzisław Śląski, Poland).

## Surface growth measurements and growth modelling

For all static experiments, time zero was defined as the time the suspension was applied to the surface of the agar plate. The diameters of developing colonies were measured at appropriate time intervals (daily or as needed) to capture the lag and exponential phases. Growth measurements were continued until the colony reached the edge of the Petri dish (i.e., fully covered the plate) or until growth ceased. Colony diameters were recorded using a Vernier calliper (150 mm  $\times$  0.02 mm; Sinochem Jiangsu, Nanjing, China) in two directions at right angles to each other, without opening the dishes. The final diameter of the colonies (expressed in micrometres) was calculated as an arithmetic mean. The maximum storage period was less than 3 months.

## Growth modelling

### Primary model

Baranyi and Roberts (1994) model within the Excel-based tool DMfit v. 3.5 was used to fit the primary growth data. A biphasic Baranyi's function was used in which the logarithmic term where  $d_{max}$  appears was deleted to omit the upper asymptote, as suggested by Valík et al. (1999).

### Secondary models

The Ratkowsky-type model (RTK) was employed to quantify the effect of water activity ( $a_w$ ) and temperature ( $T$ ) on the lag phase ( $1/\bar{\lambda}$ ):

$$\frac{1}{\bar{\lambda}} = b \cdot (a_w - a_{wmin}) \cdot (T - T_{min})^2 \quad (5)$$

where, are the parameters, and  $b$  is the regression coefficient to be calculated (Neumeier et al., 1997).

The cardinal model (CM) was used as the secondary model to describe the influence of  $T$  and  $a_w$  on the specific growth rate ( $sgr$ ):

$$sgr = sgr_{opt} \cdot CM(T) \cdot CM(a_w) \quad (6)$$

where is the optimal surface growth rate,

$$CM(T) = \left\{ \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min}) \cdot (T_{opt} - T_{min}) \cdot (T - T_{opt}) - (T_{opt} - T_{max}) \cdot [T_{opt} + T_{min} - 2T]} \right\} \quad (7)$$

and

$$CM(a_w) = \left\{ \frac{(a_w - a_{wmax})(a_w - a_{wmin})^2}{(a_{wopt} - a_{wmin}) \cdot (a_{wopt} - a_{wmin}) \cdot (a_w - a_{wopt}) - (a_{wopt} - a_{wmax}) \cdot [a_{wopt} + a_{wmin} - 2a_w]} \right\} \quad (8)$$

Except of , this secondary model includes cardinal parameters of two environmental factors with direct biological meaning, such as  $T_{min}$  (theoretical minimum temperature and water activity),  $T_{opt}$  (optimal temperature and water activity), and  $T_{max}$  (maximum temperature and water activity above which fungal growth is not likely). The parameters of the model, including their errors, were estimated with non-linear regression tools incorporated in Statistica vs. 10 (Tibco, Santa Clara, USA).

The time ( $t_3$ ) that each mycelium became visible ( $d_3 = 3$  mm) was calculated from the equation:

$$t_3 = \bar{\lambda} + \frac{d_3}{sgr} \quad (9)$$

where  $d_3$  is the minimum diameter of a visible mycelium (3 mm).

## Statistical analyses

The growth parameters ( $sgr_{opt}$ ,  $\bar{\lambda}$ , and  $d_{max}$ ) were expressed as mean values  $\pm$  standard deviation (SD). To evaluate the accuracy of both primary and secondary growth models, the coefficient of determination ( $R^2$ ) and root-mean-square error (RMSE) metrics were calculated using Microsoft Excel (Microsoft 365, Redmond, WA, USA).

## Validation

To validate the model, supplementary experiments were conducted using programmed temperature shifts in SMA medium at  $a_w$  levels of 0.992 and 0.985 (adjusted with 1 % NaCl). The time/temperature regime 25 °C and 18 °C (each for 2 days), followed by 15 °C and 10 °C (each for 5 days), simulates traditional fermentation and ripening conditions typical of Slovak artisan ewe lump cheese production (Medvedová

and Valik, 2012). The growth parameters of two-step modelling performed in this work were used to calculate colony diameters of *M. circinelloides*. Without a stationary phase, the following general equation (8) was used:

$$d_{cal} = d_0 + sgr(CM)_{cal} \cdot (t - \lambda) \tag{10}$$

where  $d_{cal}$  is a calculated colony diameter,  $d_0$  is the initial colony diameter at each  $T/t$  module, which is also the final  $d_{cal}$  of the previous  $T/t$  module.

The bias, accuracy and discrepancy factors ( $B_f$ ,  $A_f$  and %D, respectively) representing validation indices were calculated using the following equations (Baranyi et al., 1999):

$$B_f = e^{\frac{\sum_{i=1}^n (\ln y_i^{cal} - \ln y_i^{exp})}{n}} \tag{11}$$

$$A_f = e^{\sqrt{\frac{\sum_{i=1}^n (\ln y_i^{cal} - \ln y_i^{exp})^2}{n}}} \tag{12}$$

$$\%D = (A_f - 1) \cdot 100\% \tag{13}$$

where  $y_i^{cal}$  is the calculated value for the  $i$ -th observation and  $y_i^{exp}$  is the experimental value for the  $i$ -th observation.

## Results and discussion

### Primary growth of *M. circinelloides*

The surface growth parameters of *M. circinelloides* cultivated on SMA are presented in Table 1. As expected, the lag phase shortened with increasing temperature and water activity, while specific growth rate ( $sgr$ ) values increased until reaching optimal conditions, after which they declined. The average coefficients of determination exceeded 0.978, underscoring the high consistency and smoothness of growth curves observed across triplicate experiments. Root-mean-square error ( $RMSE$ ) values for the growth curves ranged from 0.73 mm to 5.17 mm, yielding an average of  $2.36 \pm 1.08$  mm. For comparison, the primary model employed by Van Long et al. (2021) similarly described radial growth with fitting quality reflected by coefficients of determination ( $R^2$ ) above 0.80 and  $RMSE$  values below 5.32 mm.

**Table 1.** Average growth parameters of *Mucor circinelloides* 1L cultivated on SMA, along with statistical indices describing growth curve consistency across triplicate experiments

$T$ (°C)	$a_w$	$sgr$ (mm/d)	$\bar{n}$ (d)	$d_{max}$ (mm)	$R^2$	$RMSE$ (mm)
6	0.992	3.62±0.02	8.00±0.28	106.2±0.8	0.996	2.41
	0.985	2.21±0.10	9.48±0.46	61.7±9.7	0.978	2.65
	0.975	ng	nl	-	-	-
8	0.992	3.50±0.14	3.22±0.28	109.4±1.7	0.989	4.20
	0.985	2.81±0.10	4.33±0.12	129.5±0.4	0.998	1.95
	0.975	ng	nl	-	-	-
12	0.992	8.04±0.14	2.91±0.12	133.0±4.8	0.999	1.21
	0.985	8.28±0.22	3.83±0.24	N/A	0.995	2.78
	0.975	ng	nl	-	-	-
15	0.992	10.97±0.46	2.00±0.17	132.9±1.7	0.998	1.53
	0.985	12.79±0.05	2.55±0.04	N/A	0.997	2.42
	0.975	2.69±0.14	2.78±0.10	21.3±0.1	0.989	0.73
18	0.992	13.13±0.17	1.85±0.04	N/A	0.999	1.09
	0.985	12.84±0.12	1.98±0.22	N/A	0.996	2.46
	0.975	6.07±0.22	6.27±0.52	N/A	0.994	3.48
21	0.992	14.69±0.82	1.38±0.09	N/A	0.994	2.58
	0.985	19.32±1.30	1.68±0.08	N/A	0.993	3.93
	0.975	6.91±0.10	5.80±0.08	N/A	0.982	5.17
25	0.992	21.17±0.31	0.85±0.02	N/A	0.999	1.10
	0.985	27.79±0.86	1.15±0.10	N/A	0.997	2.26
	0.975	14.38±0.58	1.91±0.37	N/A	0.992	3.19
30	0.992	31.82±0.31	1.18±0.05	N/A	0.996	2.51
	0.985	24.38±1.68	0.88±0.18	N/A	0.996	2.17
	0.975	22.20±0.60	1.56±0.04	N/A	0.998	1.73
33	0.992	18.77±0.22	0.70±0.01	115.6±6.5	0.998	1.47
	0.985	32.26±0.82	0.99±0.10	131.9±1.8	0.998	1.7
	0.975	23.35±0.60	1.28±0.13	N/A	0.996	2.45
35	0.992	12.62±0.41	0.47±0.10	105.0±3.2	0.990	3.92
	0.985	25.18±0.36	0.93±0.01	124.5±0.4	0.999	1.22
	0.975	13.46±0.50	1.52±0.04	96.9±1.9	0.998	1.46

$T$  - incubation temperature;  $sgr$  - surface growth rate;  $\bar{n}$  - lag phase duration; nl - no lag; ng - no growth;  $d_{max}$  - maximum colony diameter in the stationary phase; N/A - non-applicable.

## Secondary modelling

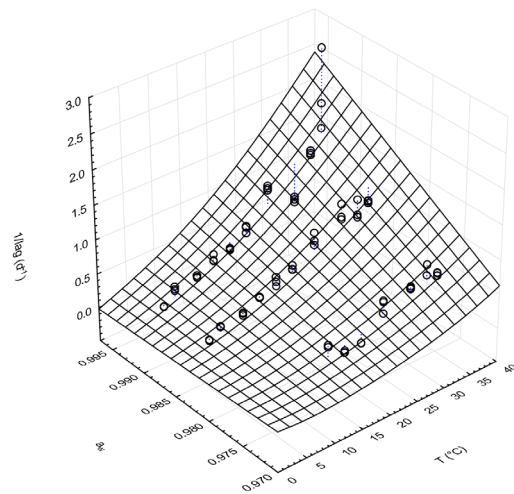
### Lag phase

To model the lag phase ( $\bar{t}$ ), reciprocal values were fitted using the Ratkowsky equation, incorporating the environmental factors, temperature ( $T$ ) and water activity ( $a_w$ ). Figure 1 displays the model's application, with estimated parameters as follows:  $b=0.031\pm 0.005$  1/°C/Öd,  $T_{min} = -7.77\pm 0.20$  °C, and  $a_{w\ min} = 0.962\pm 0.002$ . The model produced a total RMSE of 0.209 1/d and a coefficient of determination ( $R^2$ ) of 0.811, indicating a reasonably good fit. The parameter errors and associated statistical indices were deemed acceptable. For reference, the  $R^2$  reported in a prior study by Koňuchová et al. (2024) using a cardinal temperature model was similarly robust ( $R^2 = 0.716, 0.956, 0.783, \text{ and } 0.970$ ). In addition, Van Long et al. (2021) explored variability in parameters estimated using the cardinal model for radial growth in 29 *Penicillium roqueforti* strains. Their model also included  $T$  and  $a_w$  as environmental variables, yielding  $R^2$  values ranging from 0.703 to 0.990.

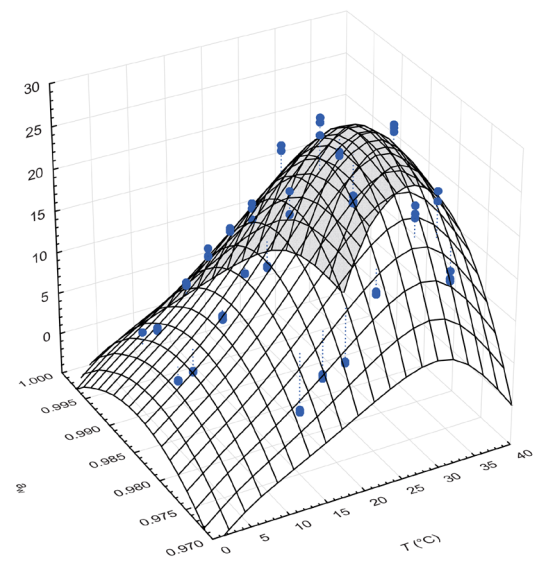
### Surface growth rate

The effect of temperature and water activity ( $a_w$ ) on the surface growth rate of *M. circinelloides* cultivated on SMA medium is depicted in Figure 2. The interaction of these environmental factors was modelled using the cardinal model, with parameter estimates summarised in Table 2. Key parameters,  $sgr_{opt} = 33$  mm/d,  $T_{opt} \approx 32$  °C, and  $a_{w\ min} = 0.97$ , indicate a high growth rate of the strain, along with sensitivity to decreasing  $a_w$  adjusted with NaCl. In contrast, the strain studied by Morin-Sardin et al. (2016) exhibited a lower  $a_{w\ min}$  of 0.927 under similar NaCl adjustments. From this perspective, the  $a_{w\ min} = 0.97$  observed in our strain might appear theoretical; however, in our supplementary experiments, no growth was detected at NaCl concentrations exceeding 3%. Despite the use of a different medium, this discrepancy likely reflects a strain-specific sensitivity to NaCl. As *M. circinelloides* belongs to lower fungi, this rapid growth aligns with expected physiological behaviour. Regarding growth rates, Morin-Sardin et al. (2016) reported  $sgr_{opt}$  values ranging from 9.3 to 19.3 mm/d for *M. circinelloides* grown on Potato Dextrose Agar (PDA). In contrast, Gougouli et al. (2011) observed a higher  $sgr_{opt}$  of 1.53 mm/h - equivalent to approximately 36.7 mm/d on Malt Extract Agar, a value closely aligned with our estimate (Table 2).

Other cardinal model parameters estimated in Table 2 also show close agreement with those reported by Gougouli et al. (2011). For instance, the theoretical  $T_{min}$  of -3.2 °C aligns well with their reported -3.0 °C, while  $T_{opt}$  of 31.7 °C and  $T_{min}$  of 35.9 °C compare favorably with their respective values of 29.0 °C and 37.0 °C. Similarly, Morin-Sardin et al. (2016) documented for *M. circinelloides* cultivated on PDA medium an almost identical  $T_{opt}$  of 29.2 °C, alongside a comparable  $T_{min}$  of -1.2 °C and a slightly higher  $T_{max}$  of 40.9 °C.



**Figure 1.** RTK secondary model depicting the effect of temperature and water activity on the reciprocal of lag phase duration ( $d^{-1}$ ) during radial growth of *Mucor circinelloides* strain 1L. The solid grid shows values fitted by Eq. 5 to the observed data (open circles). Results represent three independent biological replicates.



**Figure 2.** Effect of the temperature on the surface growth rate ( $sgr$ ) of *M. circinelloides* strain 1L cultivated on SMA medium. The Cardinal Model (solid grid) was fitted to the observed surface growth rate values (full dots)

**Table 2.** Estimated parameters of the cardinal model describing the surface growth of *Mucor circinelloides* 1L cultivated on SMA medium

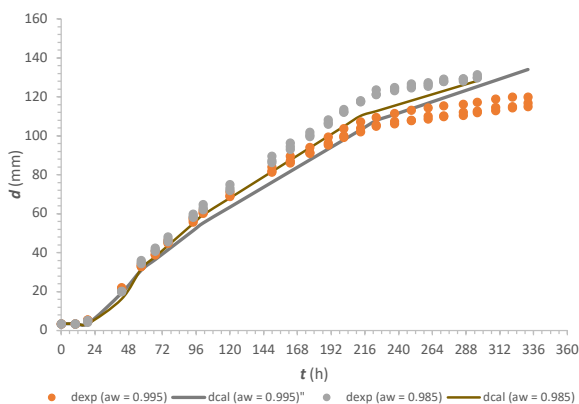
CM parameters	SE	SE of fit	$R^2$
$sgr_{opt}$ (mm/d)	33.04	1.60	0.393
$T_{min}$ (°C)	-3.18	0.04	
$T_{opt}$ (°C)	31.74	0.13	
$T_{max}$ (°C)	35.94	0.12	
$a_{w\ min}$	0.970	0.003	
$a_{w\ opt}$	0.985	0.001	
$a_{w\ max}$	1.00	fixed	

SE - standard error;  $R^2$  - coefficient of determination

### Growth model validation

Validation outcomes shown in Figure 3 express the colony diameters that were predicted modularly across the temperature/time sequence using the previously established models for surface growth rate ( $sgr$ ) and lag phase.

The statistical indices,  $RMSE$  and coefficient of determination ( $R^2$ ), for surface growth modelling of *M. circinelloides* on SMA medium at both  $a_w$  conditions were 9.35 mm and 0.989, respectively (Table 3). Despite increased variability, the progression of predicted colony diameters over time closely mirrored the experimental measurements, with high statistical significance.



**Figure 3.** Comparison of predicted colony diameter ( $d_{cal}$ ) and experimentally observed colony diameter ( $d_{exp}$ ) of *Mucor circinelloides* strain 1L cultivated on SMA medium at  $a_w$  levels of 0.995 and 0.985. Calculations were performed modularly under a dynamic temperature regime of 25 °C (48 h), 18 °C (48 h), 15 °C (120 h), and 10 °C (120 h)

**Table 3.** Statistical and validation indices for growth of *Mucor circinelloides* in SMA under dynamic temperature regimen of 25 °C (48 h), 18 °C (48 h), 15 °C (120 h), and 10 °C (120 h)

Parameter	For combined data sets at $a_w$ levels of 0.992 and 0.985	
$RMSE$	9.354	
$R^2$	0.989	
	$a_w$ 0.995	$a_w$ 0.985
$B_f$	0.977	0.926
$A_f$	1.117	1.108
$\%D_f$	-11.728	-10.818

$R^2$  - coefficient of determination;  $RMSE$  - root mean square error;  $A_f$  - accuracy factor;  $B_f$  - bias factor;  $\%D_f$  - % discrepancy.

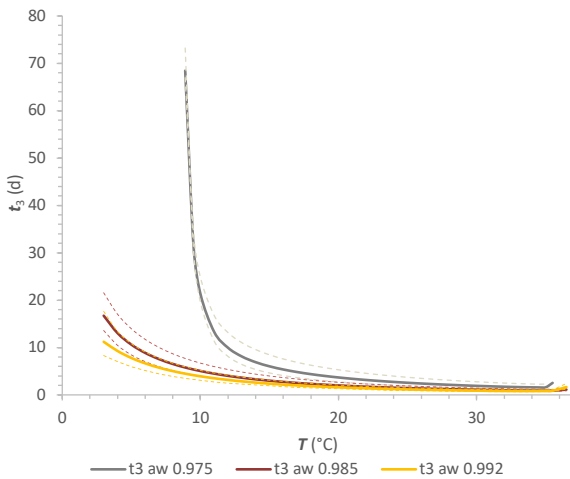
Graphical evaluation in Figure 3, along with bias ( $B_f$ ) and accuracy ( $A_f$ ) factors, further confirms acceptable model performance in predicting surface growth under dynamic temperature conditions (Burgain et al., 2013). Specifically, the calculated  $B_f$  values of 0.977 and 0.926 at  $a_w$  levels of 0.992 and 0.985 indicated 2.3 % and 7.4 % underestimation of observed colony diameters, respectively, suggesting a “fail-dangerous” tendency.

In comparison, Baert et al. (2007) modelled the growth of *Penicillium expansum* on apple purée agar using the Ratkowsky model, and reported  $B_f$  values ranging between 0.91 and 1.14, which are of similar magnitude to those observed in our study. Moreover, the  $A_f$  values of 1.117 and 1.108 indicate deviations between predicted and observed diameters of up to 11.7 % and 10.8 % (primarily underestimations). These findings are consistent with those of Judet-Correia et al. (2010), who reported  $A_f$  values of 1.11 and 1.29 for growth rate predictions of *Penicillium expansum* and *Botrytis cinerea*, respectively.

### Time required for a visible colony

Cardinal model parameters for lag phase and surface growth rate were applied to predict the time required for a visible colony to form, a valuable metric in dairy practice. Such predictions are especially relevant for fast-growing fungal species like *Mucor circinelloides*, a member of the lower fungi. In the context of microscopic fungi, a colony diameter of 3 mm ( $t_3$ ) is generally considered visible. The growth data presented in this study confirm that *M. circinelloides* is characterised by a short  $t_3$ , reflecting its rapid colonisation behaviour. Compared to *Geotrichum candidum*, commonly known as “machinery mould” (Cai and Snyder, 2019), *M. circinelloides* demonstrates faster growth. These findings are consistent with Gougouli et al. (2011), who classified *M. circinelloides* among dairy spoilage fungi exhibiting the shortest  $t_3$  values under high  $a_w$  conditions.

Based on experimental data for *M. circinelloides* strain 1L cultivated on SMA medium, the predicted  $t_3$  values illustrated in Figure 4 demonstrate a clear dependency on water activity ( $a_w$ ) and temperature. Notably, combinations of lowered  $a_w$  (<0.975) and reduced temperatures (<10 °C)



**Figure 4.** Predicted time to visible colony formation ( $t_3$ ) of *Mucor circinelloides* strain 1L cultivated on SMA medium, plotted as a function of water activity ( $a_w$ ) and temperature.

resulted in a pronounced extension of  $t_3$ , with visible colony formation delayed beyond 30 days.

## Conclusions

*Mucor circinelloides* strain 1L demonstrated rapid growth on SMA medium, consistent with the physiological traits of lower fungi and dairy-associated spoilage organisms. Ratkowsky and cardinal models effectively described lag phase and surface growth rate responses to environmental variables, with model fits confirmed by high  $R^2$  and low RMSE values. Validation under dynamic temperature regimes relevant to ewe cheese fermentation confirmed strong predictive capacity, despite slight “fail-dangerous” tendencies. Calculated  $t_3$  values indicated that under suboptimal conditions ( $a_w < 0.975$ , temperature  $< 10$  °C), visible colony formation can be delayed beyond 30 days, critical for shelf-life forecasting and spoilage risk assessment. The modelling framework provides a robust predictive tool for fungal growth behaviour in dairy contexts, facilitating informed hygiene protocols and spoilage prevention strategies.

## Research funding

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## Kinetičko modeliranje rasta *Mucor circinelloides* na agaru od obranog mlijeka pri uvjetima temperature i aktiviteta vode relevantnim za mliječnu industriju

### Sažetak

Ovim je istraživanjem ispitana kinetika površinskog rasta soja *Mucor circinelloides* 1L uzgojenog na agaru od obranog mlijeka (SMA) pri različitim temperaturama i vrijednostima aktiviteta vode ( $a_w$ ), s ciljem predviđanja kvarenja mliječnih proizvoda. Krivulje rasta prilagođene Baranyijevom modelu pokazale su visoku glatkoću i ponovljivost ( $R^2 > 0,978$ ; RMSE =  $2,36 \pm 1,08$  mm). Primarni parametri rasta, uključujući trajanje faze zastoja i površinsku brzinu rasta ( $sgr$ ), kvantificirani su u triplikatu i modelirani pomoću Ratkowskyjeva i kardinalnog pristupa. Sekundarno modeliranje otkrilo je ovisnost o okolišnim uvjetima, s optimalnom površinskom brzinom rasta ( $sgr_{opt}$ ) od 33 mm/dan te procijenjenim kardinalnim parametrima ( $T_{opt} \approx 32$  °C; teoretski  $a_{w\min} = 0,97$ ). Validacijski pokusi koji su simulirali uvjete fermentacije ovčjeg grudastog sira pokazali su visoku prediktivnu točnost ( $R^2 = 0,989$ ), pri čemu su faktori pristranosti i preciznosti potvrdili pouzdanost modela unatoč neznatno manjim vrijednostima. Predviđena vremena vidljivosti ( $t_3$ , promjer kolonije  $\geq 3$  mm) ukazala su na brzo koloniziranje u povoljnim uvjetima, dok su kombinacije sniženih temperatura ( $< 10$  °C) i niskog aktiviteta vode ( $< 0,975$ ) znatno produljile  $t_3$  iznad 30 dana. Ovi rezultati naglašavaju vrijednost kinetičkog modeliranja za procjenu rizika od gljivičnog kvarenja te potrebu za strogim higijenskim mjerama u sustavima proizvodnje mliječnih proizvoda.

**Ključne riječi:** modeliranje površinskog rasta; kardinalni model; *Mucor circinelloides*; gljivično kvarenje

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