



## Article

# Modelling the Radial Growth of *Geotrichum candidum*: Effects of Temperature and Water Activity

Martina Koňuchová \* and Ľubomír Valík

Department of Nutrition and Food Quality Assessment, Institute of Food Science and Nutrition, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinskeho 9, SK-812 37 Bratislava, Slovakia; lubomir.valik@stuba.sk

\* Correspondence: martina.konuchova@stuba.sk; Tel.: +421-2-59325-524

**Abstract:** Modelling the growth of microorganisms in relation to environmental factors provides quantitative knowledge that can be used to predict their behaviour in foods. For this reason, the effects of temperature and water activity ( $a_w$ ) adjusted with NaCl on the surface growth of two isolates and one culture strain of *Geotrichum candidum* were studied. A dataset of growth parameters obtained from almost 600 growth curves was employed for secondary modelling with cardinal models (CMs). The theoretical minimal temperature resulting from the modelling of the mycelium proliferation rate ranged from  $-5.2$  to  $-0.4$  °C. Optimal and maximal temperatures were calculated and found to have narrow ranges of 25.4 to 28.0 °C and 34.2 to 37.6 °C, respectively. Cardinal  $a_w$  values associated with radial growth ( $a_{w\ min}$  from 0.948–0.960 and  $a_{w\ opt}$  from 0.992–0.993) confirmed the salt sensitivity of the species. Model goodness-of-fit was evaluated by the coefficient of determination  $R^2$ , which ranged from 0.954 to 0.985, and RMSE, which ranged from 0.28 to 0.42. Substantially higher variability accompanied the lag time for growth modelling than the radial growth rate modelling despite the square root transformation of the reciprocal lag phase data ( $R^2 = 0.685$  to 0.808). Nevertheless, the findings demonstrate that the outputs of growth modelling can be applied to the quantitative evaluation of the roles of *G. candidum* in fresh cheese spoilage as well as the ripening of Camembert-type cheeses or various artisanal cheeses. Along with validation, the interactions with lactic acid bacteria can be included to improve the predictions of *G. candidum* in the future.

**Keywords:** *Geotrichum candidum*; surface growth modelling; cardinal model; spoilage



**Citation:** Koňuchová, M.; Valík, Ľ. Modelling the Radial Growth of *Geotrichum candidum*: Effects of Temperature and Water Activity. *Microorganisms* **2021**, *9*, 532. <https://doi.org/10.3390/microorganisms9030532>

Academic Editor: Hubert Antolak

Received: 8 February 2021

Accepted: 2 March 2021

Published: 5 March 2021

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## 1. Introduction

Food-associated microscopic fungi and cheese-contaminating mycobiota are generally known as agents of spoilage associated with mycotoxin production. Fungal spoilage of foods usually manifests as visible mycelium, a wide variety of metabolic by-products that cause off-odours and flavours and visible changes in colour or texture [1].

Species associated with the deterioration of dairy products in the agro-food industry include *Geotrichum* species [2–4]. *Geotrichum candidum* can contaminate processed foods and adversely affect their nutritional quality through physical, chemical, and sensorial changes. Having mouldy and yeasty properties, *G. candidum* significantly affects the flavour, aroma, colour and texture of fresh cheeses, butter, fermented milk, cream, poultry meat, fruit juices and vegetables [4–8].

However, another main aspect of *G. candidum* presence in foods is considered. This microscopic fungus plays positive roles in certain dairy products as it is involved in the fermentation and ripening of both industrially and artisanally produced cheeses, thus contributing to their final characteristics. For example, in some Camembert types and various Slovakian soft cheeses (ewes' lump and bryndza), *G. candidum* strains exist in commensal relationships with other fermentation or ripening microbiota. *G. candidum* assimilates lactic acid, increases pH and enables the growth of other surface microorganisms. In Camembert-type cheese, it undergoes mycelial growth, thus supporting *Penicillium*

*camemberti*, protects food surfaces against contamination and produces enzymes, all of which contribute to proper ripening [9,10]. *G. candidum* is frequently used during the maturation and flavouring of mould-ripened cheeses (Camembert, Brie, St. Marcellin), blue-veined cheeses (Danablu, Roquefort, Stilton, Gorgonzola) and smear-ripened cheeses (Limburger, Münster, Livarot, Tilsit, Reblochon, Pont-l'Évêque cheese) [6,11–15]. In the Scandinavian drink Viili, it is one of the components of the starter culture used for the fermentation of this dairy product [16]. Moreover, *G. candidum* can be found at the early stages of kefir production as it covers the kefir grain surface [17] as well as in home-made and industrial products [18].

Cheese ripening is a complex biochemical process and for *G. candidum*, it is desirable that it only participates to some extent and contributes to commensal growth and metabolic equilibrium relationships with other members of the cheese microbiota. Fungi exhibit tolerance to a wide range of pH values; the principal factors affecting fungi development are water activity ( $a_w$ ) and temperature [19,20]. Therefore, we aimed to quantify the surface growth of *G. candidum* as influenced by temperature and  $a_w$  adjusted with NaCl to provide models suitable for either the prediction of microbial spoilage of fresh cheeses or the optimization of ripening processes in dairy practice.

## 2. Materials and Methods

### 2.1. Fungal Strains and Culture Conditions

Two representative food-isolated strains and one culture strain of *G. candidum* were selected from a set of 18 isolates and 6 culture collection strains that had been evaluated with respect to growth rate variability in a previous study by Koňuchová and Valík [21]. Strains *G. candidum* G and *G. candidum* I, belonging to the collection of the Institute of Food Science and Nutrition (Slovak University of Technology in Bratislava, Slovakia), were isolated from cottage cheese and artisanal ewes' lump cheese, respectively, and *G. candidum* CBS 557.83 was obtained from the Westerdijk Fungal Biodiversity Institute (Utrecht, the Netherlands). All strains were refrigerated ( $5 \pm 1$  °C) on plate count skim milk agar (SMA; Merck, Darmstadt, Germany) slants and periodically cultured in diluted SMA agar. For long-term storage, the cultures were frozen at  $-70$  °C in tubes containing yeast malt broth (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 20% glycerol.

### 2.2. Experimental Design

A complete factorial design was set up to study the effects of NaCl and temperature. Growth trials with four replicates of each *G. candidum* strain were performed with fifty combinations of experimental conditions according to the following factor levels:

Storage temperature (°C): 6, 8, 12, 15, 18, 21, 25, 30, 34, 37

NaCl (%): 0, 1, 3, 5, 7 ( $w/v$ )

Standard growth SMA medium, which was acidified with 10 mL/L lactic acid (Sigma-Aldrich, St. Louis, MO, USA) to pH 5.5, was used in the experiments. The  $a_w$  of the medium was adjusted with 1, 3, 5 or 7% sodium chloride (Sigma-Aldrich, St. Louis, MO, USA) and measured after sterilization with Novasina LabMaster- $a_w$  (Novasina, Lachen, Switzerland). The inoculum was prepared, and the growth experiments were carried out according to Koňuchová and Valík [21].

The diameters of *G. candidum* colonies ( $d$ ) were measured using a Vernier calliper (150 × 0.02 mm, Sinochem Jiangsu, Nanjing, China) in two orthogonal directions per plate without opening the dishes, and the final values were calculated according to previous work.

### 2.3. Model Description

#### 2.3.1. Primary Model

Colony growth data were fitted using the primary growth model of Baranyi and Roberts [22] included in the in-house Excel 365 (Microsoft, Redmond, WA, USA) Add-in package 'DMFit' version 3.5 (ComBase, University of Tasmania Food Safety Centre, Hobart, Australia). In total, 600 growth curves were evaluated (representing two isolates and one

culture collection strain cultured in quadruplicate at ten incubation temperatures and five NaCl concentrations).

### 2.3.2. Secondary Modelling

The cardinal model by Rosso et al. [23] describes the effects of food environmental factors on microbial growth parameters, e.g., growth rate and lag time for growth. This model is associated with the gamma concept [24] and characterized by high “goodness of fit”; moreover, it provides four parameters that all have physiological meaning [20,25]. For *G. candidum*, the following models were used for radial growth rate (RGR) and lag time for growth ( $\lambda$ ) modelling against temperature and  $a_w$ :

$$RGR = RGR_{opt} \times CM(T) \times CM(a_w) \quad (1)$$

$$\sqrt{\frac{1}{\lambda}} = \sqrt{\frac{1}{\lambda_{opt}}} \times CM(T) \times CM(a_w) \quad (2)$$

where

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

$$CM(a_w) = \frac{(a_w - a_{w\ max})(a_w - a_{w\ min})^2}{(a_{w\ opt} - a_{w\ min}) [(a_{w\ opt} - a_{w\ min})(a_w - a_{w\ opt}) - (a_{w\ opt} - a_{w\ max})(a_{w\ opt} + a_{w\ min} - 2a_w)]} \quad (4)$$

Based on the experimental data, the cardinal temperatures ( $T_{min}$ ,  $T_{max}$ ,  $T_{opt}$ ) and  $a_w$ -values ( $a_{w\ min}$ ,  $a_{w\ max}$ ,  $a_{w\ opt}$ ) as well as optimal radial growth rate ( $RGR_{opt}$ ) and lag phase duration ( $\lambda$ ) were calculated with non-linear regression using the Excel Solver tool.

### 2.3.3. Time Required to Achieve Visible Colonies

The time values for *G. candidum* to form visible 3 mm colonies ( $t_3$ ;  $d = 3$  mm) at specific combinations of  $T$  and  $a_w$  were calculated using the following equation:

$$t_3 = \lambda + \frac{d}{RGR} \quad (5)$$

The CM models Equations (1) and (2) with the model parameters summarized in Tables 1 and 2 were used for  $\lambda$  and RGR, respectively. For the calculation of the  $t_3$ -value ranges, the CM models' coefficients were reduced or increased by the error estimated.

### 2.4. Statistical Analysis and Model Evaluation

Analysis of variance of medians was used to assess the significance of the growth conditions, colony diameter and intraspecific differences in the monitored isolates and collection strain. The results are presented as means and standard deviations. Statistical analyses were carried out using Excel.

To evaluate the goodness of fit of the predictive models, i.e., their ability to describe the observed experimental data, we used the following mathematical and statistical indices: coefficient of determination  $R^2$ , root mean square error (RMSE), mean relative error (%MRE), standard error of prediction (%SEP) and sum of squared residuals (RSS). These indices were calculated as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (Y_{obs} - Y_{pred})^2}{n - p}} \quad (6)$$

$$\%MRE = \frac{100}{n} \left( \frac{\sum_{i=1}^n |Y_{obs} - Y_{pred}|}{Y_{max} - Y_{min}} \right) \quad (7)$$

$$\%SEP = \frac{100}{\bar{\mu}_{obs}} \sqrt{\frac{\sum_{i=1}^n (Y_{obs} - Y_{pred})^2}{n}} \quad (8)$$

$$RSS = \sum_{k=1}^n (Y_{obs} - Y_{pred})_k^2 \quad (9)$$

where  $Y_{obs}$  and  $Y_{pred}$  are the observed and predicted RGR or lag time data, respectively;  $n$  is the number of experimental observations; and  $p$  is the number of model parameters [26,27].

### 3. Results and Discussion

The main advantage of the cardinal model (CM) described by Rosso et al. [23] is that it provides estimates of the cardinal values of the environmental factors affecting growth (in our case,  $T_{min}$ ,  $T_{opt}$ ,  $T_{max}$ ,  $a_{w\ min}$ ,  $a_{w\ opt}$ , and  $a_{w\ max}$ ), which are not easy to determine experimentally because fungal growth can occur several months after initial incubation [28]. Moreover, the CMs were successfully used as a predictor of the lag phase duration and/or RGR [25,29–38]. This work is based on hundreds of growth curves and provides outputs of lag time and RGR from secondary modelling. In preliminary experiments, the microscopic fungus *G. candidum* did not grow on the surface of skim milk agar at 5 °C or 38 °C over a period of more than 30 days, which is in accordance with Eliskases-Lechner et al. [8]. Thus, the mycelium growth of the two tested isolates and one collection strain was monitored at temperatures from 6 to 37 °C at intervals of 2–5 °C.

#### 3.1. Primary Surface Growth Modelling

Independently of the tested factor, the growth of *G. candidum* typically followed log-linear sigmoidal curves. The high individual coefficients of determination (with average value of  $R^2 = 0.987 \pm 0.024$ ;  $CV = 2.4\%$ ) indicated the suitability of the Baranyi model used to fit the growth curves and determine the growth parameters. Generally, slightly higher variability among  $R^2$  values was observed within quadruplicates at lower values of temperature and  $a_w$ . On the other hand, as expected for the growth parameters, significantly lower variability was recorded among growth rates than among lag phase durations, as has been reported by various authors [21,28,39–41]. The estimated values of lag phase duration ( $\lambda$ , days) and maximum RGR (mm/d) for the strains under all of the experimental conditions are summarized in Supplementary Material Table S1.

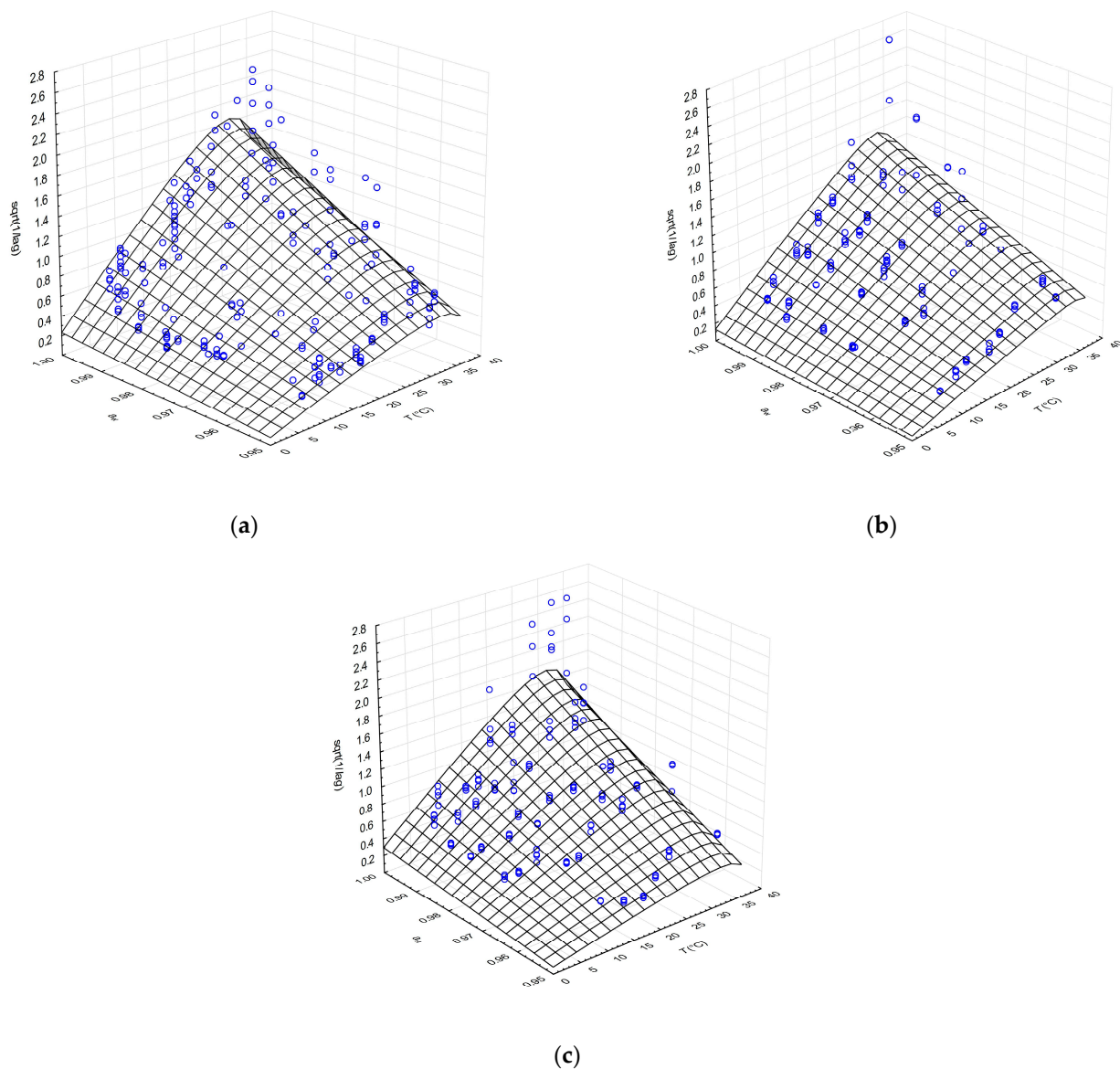
#### 3.2. Secondary Modelling

##### 3.2.1. Combined Effects of $a_w$ Adjusted with NaCl and Temperature on Lag Time

Despite the high variability of the lag time data, particularly in the optimal temperature range and higher temperatures approaching the maximal growth temperature, the CM with square root transformation of reciprocal lag values was able to describe the effects of  $a_w$  and temperature. The outputs of lag phase modelling for the cheese-isolated and CBS culture strains showed similar patterns of behaviour, as shown in Figure 1. Lag phase decreased with increasing  $a_w$  and temperature and slightly increased in the area beyond the optima, towards the maximal values of these factors (Figure 1a–c). Several qualitative radial growth studies of *Aspergillus* species, *Penicillium* species, *Fusarium oxysporum*, *Mucor circinelloides*, *Rhizopus oryzae* and *Cladosporium cladosporioides* demonstrated similar pattern of response to temperature and/or  $a_w$  on lag phase duration [28,30,42,43].

The statistical indices of the lag phase secondary modelling revealed variability around the estimated model data, with coefficient of determination ( $R^2$ ) values ranging from 0.685 to 0.808. These values are acceptable considering the many influencing factors during the population adjustment period. Relative error estimates for the individual strains were

calculated as %SEP and %MRE (using the range  $Y_{\max}-Y_{\min}$  in the denominator) and ranged between 23.3 and 32.4% and from 4.9% to 9.5%, respectively.



**Figure 1.** Effects of temperature and  $a_w$  on the lag time ( $\lambda$ ) of isolate G (a), isolate I (b) and collection strain CBS 557.83 of *G. candidum* (c) fitted with CM. Points (○) represent observed values of lag phase.

All of the parameter estimates of the CM of the lag phase were consistent across the *G. candidum* strains, except for  $1/\lambda_{\text{opt}}$ , which was accompanied by higher but acceptable errors (Table 1). Similar standard deviations of CM parameters in lag time secondary modelling were reported by Marín et al. [43] for  $a_w$  effect on *A. flavus* isolates and by Dagnas et al. [44] for temperature and  $a_w$  effect on various bakery product spoilage moulds.

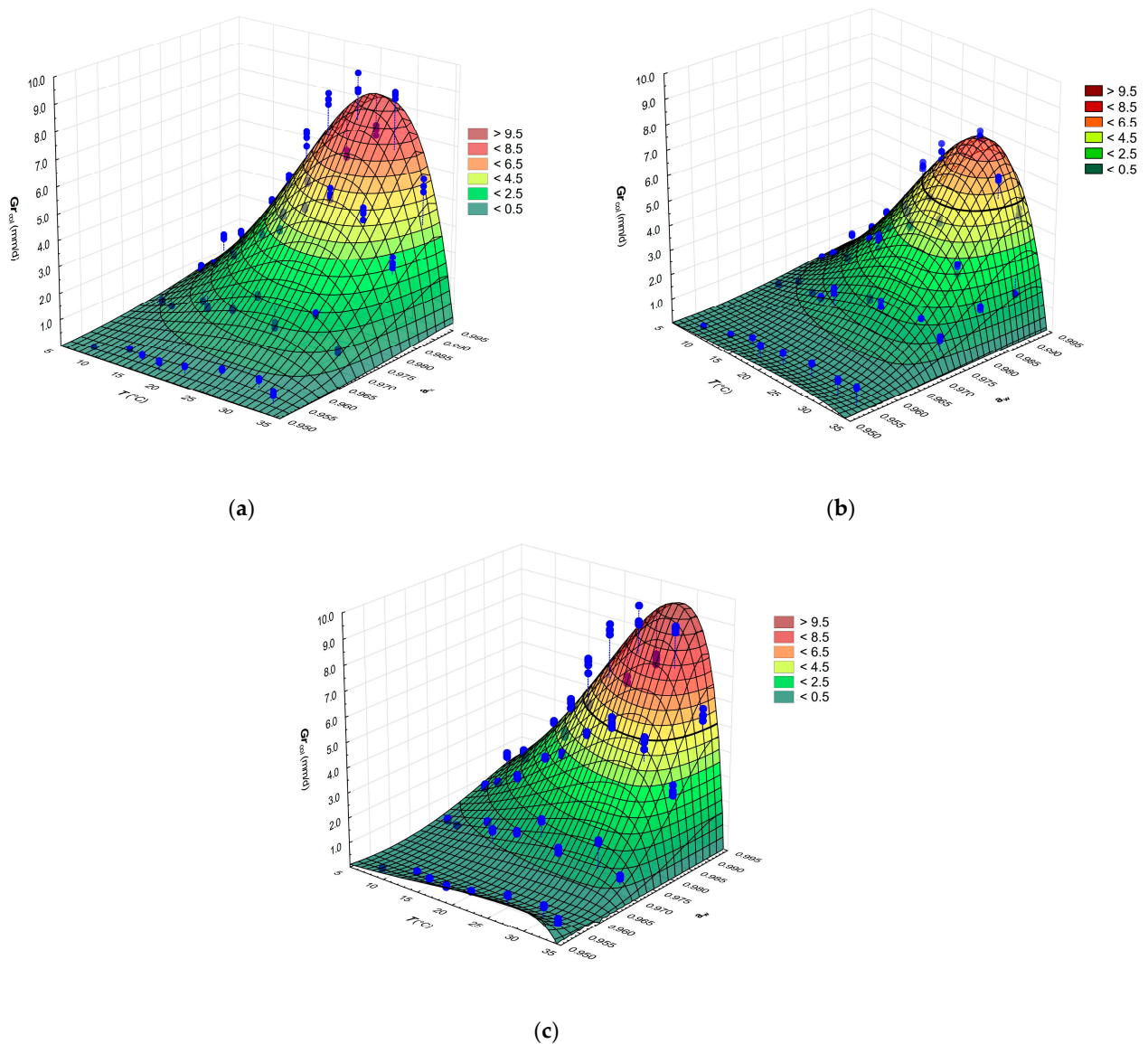
### 3.2.2. Combined Effects of $a_w$ and Temperature on the Growth of *G. candidum*

The CM was used to estimate relationships between the RGR values of the three cultures of *G. candidum* and temperature and  $a_w$  as independent variables. Figure 2a–c show that RGR initially increased with both increasing temperature and increasing  $a_w$  and the optimal range was 25–28 °C, with maximal values between 7.85 to 9.13 mm/d and a gradual decrease beyond 10 °C.

**Table 1.** Estimated cardinal model (CM) parameters and statistical indices for lag time modelling of *G. candidum* against temperature and  $a_w$ .

Parameters	Isolate G	Isolate I	Strain CBS 577.83
$1/\lambda_{opt}$ (1/d)	$3.30 \pm 0.25$	$3.64 \pm 0.68$	$2.97 \pm 1.06$
$T_{min}$ (°C) *	$-5.15 \pm 0.10$	$-5.45 \pm 0.14$	$-1.95 \pm 0.03$
$T_{opt}$ (°C)	$33.13 \pm 0.32$	$34.56 \pm 0.70$	$35.83 \pm 0.94$
$T_{max}$ (°C)	$37.35 \pm 0.09$	$37.09 \pm 0.11$	$37.06 \pm 0.27$
$a_w$ min	$0.927 \pm 0.003$	$0.938 \pm 0.003$	$0.925 \pm 0.003$
$a_w$ opt	0.998 (fixed value)	0.998 (fixed value)	0.997 (fixed value)
$a_w$ max	0.999 (fixed value)	0.999 (fixed value)	1 (fixed value)
RMSE	0.268	0.280	0.173
%MRE	9.5	7.6	4.9
%SEP	32.0	32.4	23.3
$n$	187	159	152
$R^2$	0.707	0.685	0.808

± (standard deviation); RMSE—root mean square error; %MRE—mean relative error; %SEP—standard error of prediction;  $n$ —number of data points;  $R^2$ —coefficient of determination; \*  $T_{min}$  means only theoretical  $T_{min}$  value.



**Figure 2.** Effects of temperature and  $a_w$  on the RGR of isolate G (a), isolate I (b) and collection strain CBS 577.83 of *G. candidum* (c) fitted with CM. Points (●) represent observed values of RGR.

The CM parameter values (Table 2) and 3D surface plots indicate that the two *G. candidum* cheese isolates behaved almost identically. The collection strain CBS 577.83 showed differences from the isolates, mainly in having higher values of  $T_{opt}$ ,  $T_{max}$  and  $RGR_{opt}$ . On the other hand, the mentioned collection strain was more drastically affected by reduction of  $a_w$  represented by 3% of NaCl than tested isolates of *G. candidum* at 25 °C. Decreasing the  $a_w$  level from a 0% to 7% NaCl content in the medium involved more than 14-fold reduction in  $RGR$  of collection strain *G. candidum* CBS 557.83 (Supplementary Material Table S1).

**Table 2.** Estimated CM parameters and statistical indices for radial growth rate ( $RGR$ ) modelling of *G. candidum* against temperature and  $a_w$ .

Parameters	Isolate G	Isolate I	Strain CBS 577.83
$RGR_{opt}$ (mm/d)	7.85 ± 0.15	6.87 ± 0.15	9.13 ± 0.20
$T_{min}$ (°C) *	−1.46 ± 0.01	−0.43 ± 0.01	−5.17 ± 0.05
$T_{opt}$ (°C)	25.92 ± 0.04	25.41 ± 0.04	28.03 ± 0.37
$T_{max}$ (°C)	35.63 ± 0.07	34.17 ± 0.38	37.57 ± 0.06
$a_w$ min	0.9479 ± 0.0014	0.9557 ± 0.0016	0.9591 ± 0.0012
$a_w$ opt	0.9934 ± 0.0003	0.9919 ± 0.0003	0.9916 ± 0.0002
$a_w$ max	0.9977 ± 0.0010	0.9988 ± 0.0011	0.9991 ± 0.0001
RMSE	0.415	0.405	0.278
%MRE	4.2	4.3	4.4
%SEP	16.4	20.0	19.7
$n$	192	184	188
$R^2$	0.985	0.954	0.980

± (standard deviation); RMSE—root mean square error; %MRE—mean relative error; %SEP—standard error of prediction;  $n$ —number of data points;  $R^2$ —coefficient of determination; \*  $T_{min}$  means only theoretical  $T_{min}$  value.

With some variation among the strains, the secondary models exhibited moderately good fit to the experimental data, as measured by the indices, e.g.,  $RMSE = 0.278$ – $0.415$  and  $R^2 = 0.954$ – $0.985$ . Several studies have observed that the statistical indices for effect of temperature and  $a_w$  on fungal growth were also very similar [32,45,46].

The CM values of  $T_{min}$ ,  $T_{opt}$  and  $T_{max}$  estimated for *G. candidum* growth are consistent with published data by Hudecová et al. [47,48], Domsch et al. [49], Pitt and Hocking [1] and Šípková et al. [50] regarding only the effect of temperature. However, the  $RGR_{opt}$  values estimated in our study are higher than the value of 5.98 mm/d reported by Hudecová et al. [48]. The study differences in estimated growth rate can be attributed to differences in the strains, models and number of independent variables investigated.

Regarding our cardinal  $a_w$  parameters, the estimation of each (minimal, optimal, maximal) from the  $RGR$  data was consistent across all three strains in the study. Moreover, the  $a_w$  min are consistent with those of previously published works [1,48,51] that report the ability of *G. candidum* to grow well on the surface of artificial growth medium or cheese with 5% NaCl (approximately  $a_w$  0.97). Plaza et al. [52] observed growth of *G. candidum* isolate from decayed citrus fruits at  $a_w$  0.95 (approximately 7% NaCl). However, these authors reported almost two times slower growth rates (3.5 mm/d) compared with growth of monitored *G. candidum* isolates G and I and strain CBS 557.83 at 30 °C at 0.995  $a_w$  level (unmodified  $a_w$ ). Other studies have also confirmed the salt sensitivity of *G. candidum*. Growth of *G. candidum* strains was slowed at 1–2% of NaCl on the surface growth medium [53–55]. Medium containing more than 4% [53,54] or 5–6% concentrations of salt [55] showed an inhibitory effect. These findings are in agreement with Hudecová et al. [14] and Marcellino and Benson [53] that salt sensitivity of *G. candidum* is strain dependent.

### 3.3. Prediction of the Time Required to Achieve Visible Colonies

The outputs of the growth modelling of *G. candidum* presented above can be used for several types of predictions in dairy practice. Regarding microscopic fungi, it is often of interest to estimate the time required to yield a visible (usually 3 mm) colony ( $t_3$ ; Table 3)

under a set of specific environmental conditions for several purposes [31,42,56–58], such as evaluating microbial loads of contaminants that can detrimentally affect food before the “use by” date, identifying intrinsic or extrinsic factors that prevent the surface growth of mycelia on food for a certain period and determining growth/no growth zones. For such purposes, CM parameters for rapidly growing strains or typical strains could be used. In this work, which was aimed at “machinery mould” species, i.e., indicators of the efficacy of cleaning and sanitation procedures in dairies, six sets of CM parameters (representing three strains,  $\lambda$  and  $RGR$ ) were developed and are available for prediction.

**Table 3.** Comparison of the time needed for *G. candidum* to create 3 mm colonies.

Temperature (°C)	% NaCl	$t_3$ (d)		RMSE for the $t_3$ Predictions Based on Both Cultures Data ( $n = 6$ )
		Strain CBS 557.83	Isolate G	
4	0.995	8.1 ± 0.3	9.6 ± 0.4	0.9
	0.99	8.6 ± 0.5	10.6 ± 0.2	1.1
	0.98	13.8 ± 0.6	15.9 ± 0.05	1.2
	0.97	32.3 ± 1.9	28.9 ± 0.9	2.4
5	0.995	6.6 ± 0.3	7.4 ± 0.3	0.5
	0.99	7.1 ± 0.4	8.0 ± 0.1	0.6
	0.98	11.3 ± 0.5	12.9 ± 0.04	0.6
	0.97	26.4 ± 1.5	21.9 ± 0.7	2.8
6	0.995	5.5 ± 0.2	5.9 ± 0.2	0.3
	0.99	5.9 ± 0.3	6.5 ± 0.1	0.4
	0.98	9.4 ± 0.4	9.7 ± 0.04	0.3
	0.97	22.0 ± 1.3	17.3 ± 0.5	2.8
7	0.995	4.7 ± 0.2	4.8 ± 0.2	0.2
	0.99	5.0 ± 0.3	5.3 ± 0.1	0.2
	0.98	7.9 ± 0.4	7.9 ± 0.4	0.2
	0.97	18.6 ± 1.1	14.1 ± 0.4	2.7
8	0.995	4.0 ± 0.2	4.0 ± 0.2	0.1
	0.99	4.3 ± 0.3	4.4 ± 0.1	0.2
	0.98	6.8 ± 0.3	6.6 ± 0.04	0.2
	0.97	15.9 ± 1.0	11.7 ± 0.3	2.5
9	0.995	3.5 ± 0.1	3.4 ± 0.1	0.1
	0.99	3.7 ± 0.2	3.8 ± 0.1	0.2
	0.98	5.9 ± 0.3	5.6 ± 0.04	0.2
	0.97	13.8 ± 0.8	9.9 ± 0.3	2.3
10	0.995	3.0 ± 0.1	2.9 ± 0.1	0.1
	0.99	3.2 ± 0.2	3.3 ± 0.1	0.1
	0.98	5.2 ± 0.2	4.9 ± 0.04	0.2
	0.97	12.1 ± 0.7	8.6 ± 0.2	2.1

Having the lowest growth rate and the longest lag durations among the strains, strain I could be excluded from consideration, but the question of which growth phase is crucial for achieving the shortest  $t_3$  remained for the other two strains. To obtain answers, we calculated time data for all strains and conditions tested, which supported the exclusion of strain I from the evaluation. Strain CBS 557.83 needed less or almost equal time to form visible colonies than isolate G at low temperatures and higher  $a_w$ , whereas  $a_w = 0.97$  favoured isolate G. Because of its shorter lag phase duration, strain G also yielded shorter or similar  $t_3$  data to the culture strain CBS 557.83 at moderate and higher temperatures (14–34 °C). The results indicated that the  $t_3$  is minimized when *G. candidum* displays higher growth rates, which are closely related to optimal temperature and  $a_w$  range. Other studies have also confirmed these findings [31,42,57,58].

The differences in  $t_3$  data gradually increased at  $a_w \leq 0.97$ , with lower  $t_3$  values obtained for isolate G. However, data are available for the whole ranges of temperature



and  $a_w$  values (Supplementary material Figure S1). Table 3 presents the lowest  $t_3$  values for *G. candidum* that can be used for prediction in fresh dairy products in the temperature range 4–10 °C, at  $a_w > 0.97$  and at pH close to 5.5. As *G. candidum* can assimilate lactic acid and increase pH, e.g., by an average of 0.2 during the culture periods in this work, we speculate that data for a broad range of pH values are relevant.

#### 4. Conclusions

The present work provides useful data for understanding the growth behaviour of *G. candidum* by considering the food environmental factors, temperature and  $a_w$ . The inclusion of multiple strains of *G. candidum*, a “machinery mould”, in this study provided information on the variability of the model outputs that are essential for specific applications in food practice. Regarding surface growth, the time necessary to achieve visible colonies can be predicted for various values throughout the temperature and  $a_w$  ranges. This prediction can be applied to various dairy products from pasteurized milk but is mainly applicable to artisanal or traditional soft or short-ripened cheeses produced from raw milk. The sensitivity of the strains to NaCl (lowering  $a_w$ ) seems to be the key element resulting from this work that can be applied to control growth of *G. candidum* at the cheese surface. Thus, for example, further experiments with controlled dry salting of cheese curd are needed to provide a validation study for cheese practice in the future.

On the other hand, for industrial fresh cheese practice, the prediction models incorporated in the Monte Carlo simulation may assist in development of spoilage-prevention strategies and product shelf-life estimations. Taking the mould prevalence in the cheese packaging during production into account, together with input model data distributions, the probability as well as uncertainty of visible contaminated production can be quantified during the storage (or period of shelf life).

Another challenge is the application of other lactic acid bacteria starters or adjunct protection cultures and incorporating their interactions with *G. candidum* into stochastic simulations. This would be necessarily associated with the further studies on activity and design of protection LAB cultures suitable for the mould(s) control.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2076-2607/9/3/532/s1>, Figure S1: Effects of temperature and  $a_w$  on the time needed for *G. candidum* to create 3 mm colonies, Table S1: The average surface growth parameters of the *G. candidum* isolates G and I and strain CBS 557.83 on SMA agar.

**Author Contributions:** Conceptualization, M.K. and L.V.; methodology, M.K. and L.V.; validation, L.V.; formal analysis, M.K.; investigation, M.K.; writing—original draft preparation, M.K.; writing—review and editing, L.V.; visualization, L.V.; supervision, L.V.; funding acquisition, L.V. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Slovak Research and Development Agency, grant number APVV-19-0031 and the Scientific Grant Agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic and Slovak Academy of Sciences, grant number VEGA 1/0532/18.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The data presented in this study are available upon reasonable request from the corresponding author.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### References

1. Pitt, J.I.; Hocking, A.D. *Fungi and Food Spoilage*, 3rd ed.; Springer Science+Business Media: New York, NY, USA, 2009; pp. 53–142.
2. Deák, T. *Handbook of Food Spoilage Yeast*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2008; pp. 2–13.
3. Hudecová, A.; Valík, L.; Liptáková, D. Effect of lactic acid bacteria on growth dynamics of *Geotrichum candidum* during co-culture in milk. *Slovak J. Food Sci.* **2010**, *4*, 387–395.

4. Laurenčík, M.; Sulo, P.; Sláviková, E.; Piecková, E.; Seman, M.; Ebringer, L. The diversity of eukaryotic microbiota in the traditional Slovak sheep cheese—Bryndza. *Int. J. Food Microbiol.* **2008**, *127*, 176–179. [[CrossRef](#)] [[PubMed](#)]
5. Boutrou, R.; Guéguen, M. Interests in *Geotrichum candidum* for cheese technology. *Int. J. Food Microbiol.* **2005**, *102*, 1–20. [[CrossRef](#)] [[PubMed](#)]
6. Koňuchová, M.; Lehotová, V.; Šípková, A.; Piecková, E.; Valík, L. Biodiversity Evaluation of *Geotrichum candidum* Link. Is arthrosporic nucleus number in *Geotrichum candidum* related to the fungus biodiversity? *Sci. Agric. Bohemica* **2016**, *47*, 181–186. [[CrossRef](#)]
7. Kornacki, J.L.; Flowers, R.S.; Bradley, R.L. Microbiology of butter and related products. In *Applied Dairy Microbiology*, 2nd ed.; Marth, E.H., Steele, J.L., Eds.; Marcel Dekker: New York, NY, USA, 2001; pp. 127–150.
8. Eliskases-Lechner, F.; Guéguen, M.; Panoff, J.M. *Geotrichum candidum*. In *Encyclopedia of Dairy Sciences*, 2nd ed.; Fuquay, J.W., Fox, P.F., McSweeney, P.L.H., Eds.; Academic Press: London, UK, 2011; Volume 4, pp. 765–771.
9. Aziza, M.; Couriol, C.; Amrane, A.; Boutrou, R. Evidences for synergistic effects of *Geotrichum candidum* on *Penicillium camembertii* growing on cheese juice. *Enzym. Microb. Technol.* **2005**, *37*, 218–224. [[CrossRef](#)]
10. Boutrou, R.; Kerriou, L.; Gassi, J.-Y. Contribution of *Geotrichum candidum* to the proteolysis of soft cheese. *Int. Dairy J.* **2006**, *16*, 775–783. [[CrossRef](#)]
11. Cogan, T.M.; Goerges, S.; Gelsomino, R.; Larpin, S.; Hohenegger, M.; Bora, N.; Jamet, E.; Rea, M.C.; Mounier, J.; Vancanneyt, M.; et al. Biodiversity of the surface microbial consortia from Limburger, Reblochon, Livarot, Tilsit, and Gubbeen cheeses. *Microbiol. Spectrum*. **2014**, *2*, 1–28.
12. Desmaures, N. Mold-Ripened Varieties. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortello, M.-R., Eds.; Academic Press: Cambridge, UK, 2014; pp. 409–416.
13. Grygier, A.; Majcher, M.; Myszkka, K. *Galactomyces geotrichum*—moulds from dairy products with high biotechnological potential. *Acta Sci. Pol. Technol. Aliment.* **2017**, *16*, 5–16. [[CrossRef](#)]
14. Hudecová, A.; Valík, L.; Liptáková, D. Quantification of *Geotrichum candidum* growth in co-culture with lactic acid bacteria. *Czech J. Food Sci.* **2009**, *27*, 18–27. [[CrossRef](#)]
15. Šipošová, P.; Koňuchová, M.; Valík, L.; Medved'ová, A. Growth dynamics of lactic acid bacteria and dairy microscopic fungus *Geotrichum candidum* during their co-cultivation in milk. *Food Sci. Technol. Int.* **2020**, in press.
16. Bakry, A.M.; Campelo, P.H. Mini-review on functional characteristics of viili and manufacturing process. *J. Food Biotechnol. Res.* **2018**, *2*, 1–6.
17. Witthuhn, R.C.; Schoeman, T.; Britz, T.J. Characterisation of the microbial population at different stages of Kefir production and Kefir grain mass cultivation. *Int. Dairy J.* **2005**, *15*, 383–389. [[CrossRef](#)]
18. Kazou, M.; Grafakou, A.; Tsakalidou, E.; Georgalaki, M. Zooming into the microbiota of home-made and industrial kefir produced in Greece using classical microbiological and amplicon-based metagenomics analyses. *Front. Microbiol.* **2021**, *12*, 621069. [[CrossRef](#)] [[PubMed](#)]
19. Dantigny, P.; Marín, S.; Beyer, M.; Magan, N. Mould germination: Data treatment and modelling. *Int. J. Food Microbiol.* **2007**, *114*, 17–24. [[CrossRef](#)] [[PubMed](#)]
20. Garcia, D.; Ramos, A.J.; Sanchis, V.; Marín, S. Predicting mycotoxins in foods: A review. *Food Microbiol.* **2009**, *26*, 757–769. [[CrossRef](#)]
21. Koňuchová, M.; Valík, L. Evaluation of radial growth dynamics variability of *Geotrichum candidum*: A quantitative approach. *J. Food Nutr. Res.* **2017**, *56*, 155–166.
22. Baranyi, J.; Roberts, T.A. Mathematics of predictive food microbiology. *Int. J. Food Microbiol.* **1995**, *26*, 199–218. [[CrossRef](#)]
23. Rosso, L.; Lobry, J.R.; Flandrois, J.P. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *J. Theor. Biol.* **1993**, *162*, 447–463. [[CrossRef](#)]
24. Zwietering, M.H.; Wiltjes, T.; De Wit, J.C.; van't Riet, K. A decision support system for prediction of the microbial spoilage in foods. *J. Food Protect.* **1992**, *55*, 973–979. [[CrossRef](#)]
25. Rosso, L.; Robinson, T.P. A cardinal model to describe the effect of water activity on the growth of moulds. *Int. J. Food Microbiol.* **2001**, *63*, 265–273. [[CrossRef](#)]
26. Pérez-Rodríguez, F.; Valero, A. *Predictive Microbiology in Foods*, 5th ed.; Springer Science+Business Media: New York, NY, USA, 2013; pp. 25–55.
27. Rosso, L.; Lobry, J.R.; Bajard, S.; Flandrois, J.P. Convenient model to describe the combined effects of temperature and pH on microbial growth. *Appl. Environ. Microbiol.* **1995**, *61*, 610–616. [[CrossRef](#)]
28. Gougouli, M.; Koutsoumanis, K.P. Modelling growth of *Penicillium expansum* and *Aspergillus niger* at constant and fluctuating temperature conditions. *Int. J. Food Microbiol.* **2010**, *140*, 254–262. [[CrossRef](#)] [[PubMed](#)]
29. Greco, M.; Pardo, A.; Pose, G.; Patriarca, A. Effect of water activity and temperature on the growth of *Eurotium* species isolated from animal feeds. *Rev. Iberoam. Micol.* **2018**, *35*, 39–48. [[CrossRef](#)]
30. Dagnas, S.; Gougouli, M.; Onno, B.; Koutsoumanis, K.P.; Membré, J.-M. Quantifying the effect of water activity and storage temperature on single spore lag times of three moulds isolated from spoiled bakery products. *Int. J. Food Microbiol.* **2017**, *240*, 75–84. [[CrossRef](#)] [[PubMed](#)]

31. Huchet, V.; Pavan, S.; Lochardet, A.; Divanac'h, M.L.; Postollec, F.; Thuault, D. Development and application of a predictive model of *Aspergillus candidus* growth as a tool to improve shelf life of bakery products. *Food Microbiol.* **2013**, *36*, 254–259. [[CrossRef](#)] [[PubMed](#)]
32. Astoreca, A.; Vaamonde, G.; Dalcero, A.; Ramos, A.J.; Marín, S. Modelling the effect of temperature and water activity of *Aspergillus flavus* isolates from corn. *Int. J. Food Microbiol.* **2012**, *156*, 60–67. [[CrossRef](#)]
33. Samapundo, S.; Devlieghere, F.; Geeraerd, A.H.; De Meulenaer, B.; Van Impe, J.F.; Debevere, J. Modelling of the individual and combined effects of water activity and temperature on the radial growth of *Aspergillus flavus* and *A. parasiticus* on corn. *Food Microbiol.* **2007**, *24*, 517–529. [[CrossRef](#)] [[PubMed](#)]
34. Panagou, E.Z.; Chelonas, S.; Chatzipavlidis, I.; Nychas, G.-J.E. Modelling the effect of temperature and water activity on the growth rate and growth/no growth interface of *Byssoschlamys fulva* and *Byssoschlamys nivea*. *Food Microbiol.* **2010**, *27*, 618–627. [[CrossRef](#)] [[PubMed](#)]
35. Nevarez, L.; Vasseur, V.; Le Madec, A.; Le Bras, M.; Coroller, L.; Leguérinel, I.; Barbier, G. Physiological traits of *Penicillium glabrum* strain LCP 08.5568, a filamentous fungus isolated from bottled aromatised mineral water. *Int. J. Food Microbiol.* **2009**, *130*, 166–171. [[CrossRef](#)]
36. Baert, K.; Valero, A.; De Meulenaer, B.; Samapundo, S.; Ahmed, M.M.; Bo, L.; Debevere, J.; Devlieghere, F. Modelling the effect of temperature on the growth rate and lag phase of *Penicillium expansum* in apples. *Int. J. Food Microbiol.* **2007**, *118*, 139–150. [[CrossRef](#)] [[PubMed](#)]
37. Parra, R.; Magan, N. Modelling the effect of temperature and water activity on growth of *Aspergillus niger* strains and applications for food spoilage moulds. *J. Appl. Microbiol.* **2004**, *97*, 429–438. [[CrossRef](#)]
38. Sautour, M.; Rouget, A.; Dantigny, P.; Divies, C.; Bensoussan, M. Application of Doehlert design to determine the combined effects of temperature, water activity and pH on conidial germination of *Penicillium chrysogenum*. *Int. J. Food Microbiol.* **2001**, *91*, 900–906. [[CrossRef](#)]
39. Marín, S.; Cuevas, D.; Ramos, A.J.; Sanchis, V. Fitting of colony diameter and ergosterol as indicators of food borne mould growth to known growth models in solid medium. *Int. J. Food Microbiol.* **2008**, *121*, 139–149. [[CrossRef](#)]
40. Sant'ana, A.; Franco, B.D.G.M.; Schaffner, D.W. Modeling the growth rate and lag time of different strains of *Salmonella enterica* and *Listeria monocytogenes* in ready-to-eat lettuce. *Food Microbiol.* **2012**, *30*, 267–273. [[CrossRef](#)]
41. Valík, L.; Medved'ová, A.; Liptáková, D. Characterization of the growth of *Lactobacillus rhamnosus* GG in milk at suboptimal temperatures. *J. Food Nutr. Res.* **2008**, *47*, 60–67.
42. Gougouli, M.; Kalantzi, K.; Beletsiotis, E.; Koutsoumanis, K.P. Development and application of predictive models for fungal growth as tools to improve quality control in yogurt production. *Food Microbiol.* **2011**, *28*, 1453–1462. [[CrossRef](#)]
43. Marín, S.; Colom, C.; Sanchis, V.; Ramos, A.J. Modelling of growth of aflatoxigenic *A. flavus* isolates from red chilli powder as a function of water availability. *Int. J. Food Microbiol.* **2009**, *128*, 491–496. [[CrossRef](#)]
44. Dagnas, S.; Onno, B.; Membéré, J.M. Modeling growth of three bakery product spoilage molds as a function of water activity, temperature and pH. *Int. J. Food Microbiol.* **2014**, *186*, 95–104. [[CrossRef](#)] [[PubMed](#)]
45. Belbahi, A.; Leguerinel, I.; Méot, J.-M.; Loiseau, G.; Madani, K.; Bohuon, P. Modelling the effect of temperature, water activity and carbon dioxide on the growth of *Aspergillus niger* and *Alternaria alternata* isolated from fresh date fruit. *J. Appl. Microbiol.* **2016**, *121*, 1685–1698. [[CrossRef](#)] [[PubMed](#)]
46. Samapundo, S.; Devlieghere, F.; De Meulenaer, B.; Debevere, J. Growth kinetics of cultures from single spores of *Aspergillus flavus* and *Fusarium verticillioides* on yellow dent corn meal. *Food Microbiol.* **2007**, *24*, 336–345. [[CrossRef](#)] [[PubMed](#)]
47. Hudecová, A.; Valík, L.; Liptáková, D. Influence of temperature on the surface growth of *Geotrichum candidum*. *Acta Chim. Slovaca.* **2009**, *2*, 75–87.
48. Hudecová, A.; Valík, L.; Liptáková, D. Surface growth of *Geotrichum candidum*: Effect of the environmental factors on its dynamics. *Slovak J. Food Sci.* **2011**, *5*, 17–22. [[CrossRef](#)]
49. Domsch, K.H.; Gams, W.; Anderson, T.-H. *Compendium of Soil Fungi*, 1st ed.; Academic Press: London, UK, 1980; Volume 2, pp. 1–859.
50. Šípková, A.; Valík, L.; Čížniar, M.; Liptáková, D. Characterization of mutual relations between *Geotrichum candidum* and *Lactobacillus rhamnosus* GG in milk: A quantitative approach. *Food Sci. Technol. Int.* **2014**, *20*, 23–31. [[CrossRef](#)] [[PubMed](#)]
51. Tornadijo, M.E.; Fresno, J.M.; Sarmiento, R.M.; Carballo, J. Study of the yeasts during the ripening process of Armada cheeses from raw goat's milk. *Le Lait* **1998**, *78*, 647–659. [[CrossRef](#)]
52. Plaza, P.; Usall, J.; Teixidó, N.; Viëas, I. Effect of water activity and temperature on germination and growth of *Penicillium digitatum*, *P. italicum* and *Geotrichum candidum*. *J. Appl. Microbiol.* **2003**, *94*, 549–554. [[CrossRef](#)] [[PubMed](#)]
53. Marcellino, S.N.; Benson, D.R. The good, the bad, and the ugly: Tales of mold-ripened cheese. In *Cheese and Microbes*, 1st ed.; Donnelly, C.W., Ed.; ASM Press: Washington, DC, USA, 2014; pp. 95–132.
54. Tempel, T.; Nielsen, M.S. Effects of atmospheric conditions, NaCl and pH on growth and interactions between moulds and yeasts related to blue cheese production. *Int. J. Food Microbiol.* **2000**, *57*, 193–199. [[CrossRef](#)]
55. Uraz, T.; Özer, B.H. Molds Employed in Food Processing. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortello, M.-R., Eds.; Academic Press: Cambridge, UK, 2014; pp. 522–528.
56. Dantigny, P. Applications of predictive modeling techniques to fungal growth in foods. *Curr. Opin. Food Sci.* **2021**, *38*, 86–90. [[CrossRef](#)]

- 
57. Aldars-García, L.; Sanchis, V.; Ramos, A.J.; Marín, S. Time-course of germination, initiation of mycelium proliferation and probability of visible growth and detectable AFB1 production of an isolate of *Aspergillus flavus* on pistachio extract agar. *Food Microbiol.* **2017**, *64*, 104–111. [[CrossRef](#)]
  58. Burgain, A.; Bensoussan, M.; Dantigny, P. Effect of inoculum size and water activity on the time to visible growth of *Penicillium chrysogenum* colony. *Int. J. Food Microbiol.* **2013**, *163*, 180–183. [[CrossRef](#)]