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Influence of temperature on the surface growth of *Geotrichum candidum*

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Abstract

Growth of filamentous fungus *G. candidum* was analysed on the surface of skim milk agar as a function of incubation temperature (5 – 37 °C). Radial growth rates of fungus were estimated using growth model of Baranyi which describes the colony diameter as a function of cultivation time. Both Ratkowsky and transformation of G-model were used as secondary models in order to describe the effect of temperature on the radial growth rate of the fungus. Both models proved to be good predictors of *G. candidum* surface growth within experimental conditions. The bias and accuracy factors were 1.007 and 1.109 for Ratkowsky model and 1.000 and 1.177 for transformed G-model. In presented work also t_3 model was proposed which allows the calculation of time needed to reach a colony diameter of 3 mm. This time is important mainly for the practical reasons because such colony would be visible and growth of fungus would be considered as spoilage in specific types of milk products. Due to ubiquitous nature of the fungus and because of its ability to grow at refrigerator temperatures the quantitative results are valuable in respect to the prevention of food spoilage.

Introduction

Geotrichum candidum is a ubiquitous filamentous yeast-like fungus that could be isolated from various habitats such as soil, water, milk, milk products, plants, fruits, insects, man and other mammals (Kocková-Kratochvílová 1990; Boutrou and Guéguen 2005; Pottier et al. 2008). *G. candidum* is recognized as a real milk mould as it was isolated from milk as early as

1850 (Wouters et al. 2002). It is an anamorphic microorganism included in the *Hemiascomycetes* and according to latest taxonomy revision the teleomorphic state of the fungus belongs to the genus *Galactomyces* Redhead and Malloch (Pottier et al. 2008). *G. candidum* creates slight felt colonies on the solid surfaces and forms such peels also on the liquid media (Kocková-Kratochvílová 1990). Body of fungus is created by branched hyphae which divide at their tips into rectangular arthric conidia.

According to Boutrou and Guéguen (2005), *G. candidum* can grow at temperature ranging from 5 to 38 °C and within the large pH interval from 3 to 11. It is well-known that this fungus is particularly sensitive to salt (Tornadijo et al. 1998) however; its sensitivity is strain dependent. *G. candidum* grows as well in the microaerophilic conditions, because it is very resistant to reduced oxygen and elevated carbon dioxide levels. The cultivation in an oxygen poor atmosphere is accompanied with an elongation of the hyphae and a loss of the lateral branches (Boutrou and Guéguen 2005).

Strains of *G. candidum* do not assimilate lactose, but are able to catabolize the lactic acid. Lactate metabolism is typical for *G. candidum* strains which were isolated from dairy products (Marcellino et al. 2001). The fungus is characterized by competitive growth especially in dairy environment. Boutrou and Guéguen (2005) determined its generation time as short as 1.1 h at 30 °C in liquid medium. Based on this fact, they stated that *G. candidum* was able colonize the surface-ripened cheeses in the early stages of ripening. Of course, their opinion is right but a lack of data on surface growth dynamics of this fungus were missing supporting this statement. That is why; one of the aims of our work was to provide surface growth data of *Geotrichum candidum*.

Common presence of *G. candidum* in raw milk results to its natural occurrence in raw milk cheeses (Erdogan et al. 2003; Godič Torkar and Vengušt 2008) produced also from goats' and ewes' milk (Tornadijo et al. 1998; Cosentino et al. 2001; Fadda et al. 2004; Hayaloglu and Kirbag 2007). On the other hand, the presence of *G. candidum* in cheeses produced from pasteurized milk is a consequence of materials re-contamination from the environment of the plant (Cosentino et al. 2001; Görner and Valík 2004; Godič Torkar and Vengušt 2008). Positive action of *G. candidum* is widely used during the production of surface-ripened cheeses. The fungus is able to increase the pH of cheese what permitted the development of next ripening organisms like *P. camemberti* and *Brevibacterium linens*. Presence of *G. candidum* could also inhibit the growth of undesirable microorganisms and

fungal contaminants (Lecocq and Gueguen 1994; Nielsen et al. 1998; Wouters et al. 2002). *G. candidum* is able to produce also D-3-phenyllacetic acid, which inhibits the growth of *Listeria monocytogenes* (Dieuleveux et al. 1998). During ripening of cheese, the fungus produces enzymes for the breakdown of protein and fat (Boutrou et al. 2006). Released fatty acids and peptides and their further catabolism lead to the development of distinctive flavours' qualities (Marcellino et al. 2001).

G. candidum like other fungi is also detrimental for some types of food and acts like spoilage agent which causes economic looses. This fungus is responsible for the degradation of fresh cheeses, fruit juices and vegetables (Laurenčík et al. 2008). In fresh cheeses like cottage cheese and quark *G. candidum* is considered as a contaminant imparting off-flavours to these products (Boutrou and Guéguen 2005). Species of genus *Geotrichum* cause the spoilage of some cream cheeses (Chapman and Sharpe 1990) and are able to grow on the surface of butter, cream and cream products (Varnam and Sutherland 1994).

In order to keep the quality of these products at sufficient level there is a need to predict the growth ability of *G. candidum*. Modelling methods developed originally for the prediction of bacterial growth could be used satisfactory also for fungal growth. Therefore, the main objective of this study was to analyse the growth potential of *G. candidum* on the surface of the solid milk medium and to apply known models to predict its growth in relation to incubation temperature. Obtained results could provide valuable information concerning the fungus ability to grow in real food with respect to environmental conditions and may contribute to the general knowledge in predictive mycology.

Material and Methods

Microorganism. The strain of *G. candidum* was isolated from ewes' lump cheese on glucose-yeast extract-chloramphenicol agar (GYCA, Imuna, Šarišské Michaľany, Slovakia) at 25 °C. The morphological identification to species level was confirmed by Dr. E. Piecková (Slovak Medical University, Bratislava, Slovakia). Strain of *G. candidum* was maintained on slopes of skim milk agar (SMA, Merck, Darmstadt, Germany) at 5±1 °C.

Inoculation and cultivation conditions. We studied growth dynamics of *G. candidum* on the surface of skim milk agar. The pH of agar was adjusted at 7.0. Three hundred milliliters of the medium were sterilized (120 °C, 20 min) and then poured into Petri dishes with internal diameter of 11 cm. After gelling, the surface of the medium was inoculated with fungus

mycelium grown on slope of SMA agar after 48-72 h. It was transferred to the centre of each Petri dish by touching the surface of agar with a microbiological loop. The Petri dishes were incubated upside down at temperatures ranging from 5 to 37 °C. At each temperature the experiments were carried out in triplicates. The colony diameter was measured in relevant time intervals using vernier caliper (150x0.02 mm, Jiangsu S. Ltd) in two orthogonal directions. The final diameter of colonies was calculated as the arithmetic mean.

Determination of pH. The pH of agar was measured using pH meter Knick Portamess equipped with sticking pH electrode Knick SE 104 (Knick, Berlin, Germany).

Primary modelling. Diameter of *G. candidum* colonies as a function of time at each temperature was modelled according to Baranyi et al. (1993). Acquired radial growth rate was subjected to the second part of microbial modelling.

Secondary modelling. Radial growth rate obtained from primary analysis was modelled as a function of temperature using two following models. The Ratkowsky model:

$$\sqrt{g} = b(T - T_{\min}) \tag{1}$$

where b (mm $h^{-0.5}$ °C^{-1}) is the regression coefficient, $T_{\min}$ (°C) is the theoretical minimum growth temperature and g (mm h^{-1}) is the radial growth rate of a microorganism.

The transformation of G-model (Gibson et al. 1994) was used as a second model. First, we applied the transformation of temperature T:

$$T_w = \sqrt{T_{\max} - T} \tag{2}$$

where $T_{\max}$ is the maximal growth temperature. Next, the following quadratic function was used for fitting the experimental data:

$$\ln g = C_0 + C_1 T_w + C_2 T_w^2 \tag{3}$$

The optimum value of T_w at which the growth rate achieved maximum value was calculated as:

$$T_w(opt) = -\frac{C_1}{2C_2} \quad (4)$$

Prediction for the radial growth rate at a given temperature T was obtained in the following way (Gibson et al. 1994, Valík and Piecková 2001):

1. calculation of T_w from temperature T using Eq. (2);
2. calculation of ln g using Eq. (3);
3. g = exp (ln g) was predicted growth rate.

Validation of the growth parameters. The secondary models (Eq. 1 and 3) were validated according to Baranyi et al. (1999). The accuracy factor, bias factor and percentage of discrepancy were calculated using following equations:

$$A_f = exp\left(\sqrt{\frac{\sum_{k=1}^{n}(\ln f(g^k) - \ln g^k)^2}{n}}\right) \quad (5)$$

$$B_f = exp\left(\frac{\sum_{k=1}^{n}(\ln f(g^k) - \ln g^k)}{n}\right) \quad (6)$$

$$D_f = (A_f - 1).100\% \quad (7)$$

where g is radial growth rate obtained from the growth curve, $f(g^k)$ – radial growth rate calculated from the model f that fitted experimental values, n – number of measurements, A_f – accuracy factor, B_f – bias factor and D_f – percentage discrepancy.

The mean square error (MSE) was calculated as follows:

$$MSE = \frac{RSS}{n} = \frac{\Sigma(g_{observed} - g_{predicted})^2}{n} \quad (8)$$

where n is the number of data points, RSS is residual sum of square.

Results and Discussion

Growth of G. candidum on the surface of SMA agar

Investigation of *G. candidum* growth on the surface of SMA agar was performed at 5, 10, 12, 15, 18, 20 (Fig. 1a), 25, 30, 35 and 37 °C (Fig. 1b). Interval of temperature was selected in order to detect the entire growth ability of the fungus. In the majority of experiments the lag-phase was absent. This was likely caused by straight inoculation of growing mycelium on the surface of the same culture medium.

At the lowest temperature the fungus was able to grow but with low radial growth rate (Tab. 1) and after two months it reached only 20 mm colonies (not shown). This result confirms the fungus capacity to grow at refrigerator temperatures (Boutrou and Guéguen 2005) what finally can lead to the spoilage of fresh cheeses even if they are adequately stored. Therefore, the maximal attention must be paid in the prevention of product contamination along the entire production chain.

Increase of incubation temperature (10 °C) resulted in the increase of radial growth rate (g = 1.31 mm d^{-1}) and fungus reached stationary phase after 6.4 weeks. Some references concerning radial growth rate of *G. candidum* on different media are available in literature. *G. candidum* growth rate of 1.63 mm d^{-1} was reported on the surface of orange serum agar with pH adjusted at 5.5 and a_w value of 0.995 (Plaza et al. 2004). In the study of van den Tempel and Nielsen (2000) the radial growth rates of two *G. candidum* strains reached 3.38 mm d^{-1} and 4.31 mm d^{-1} on the surface of cheese agar at the same temperature. As can bee seen, the growth rate of *G. candidum* varied depending on the used strain and on the design of the experiment.

At temperatures from 12 to 25 °C, the growth rate of the fungus increased with incubation temperature and the time in which *G. candidum* reached stationary phase decreased. Maximal growth rate was observed at 25 °C (g = 0.216 mm h^{-1}) and exponential growth of the fungus lasted 2 weeks. The maximal growth rate was by 67 % higher than that observed at 12 °C. Plaza et al. (2004) observed the growth rate of the fungus 4 mm d^{-1} at 25 °C what represents 23 % difference in comparison with our results.

Further temperature increase did not lead to the increase of growth rate but the decceleration was recorded. At 30 °C, the growth of *G. candidum* was about 14 % slower than at 25 °C, and at 37 °C the growth rate of the fungus achieved only 0.06 mm d^{-1}. According to

Boutrou and Guéguen (2005) the maximal temperature for growth of *G. candidum* is 38 °C what is consistent with our results.

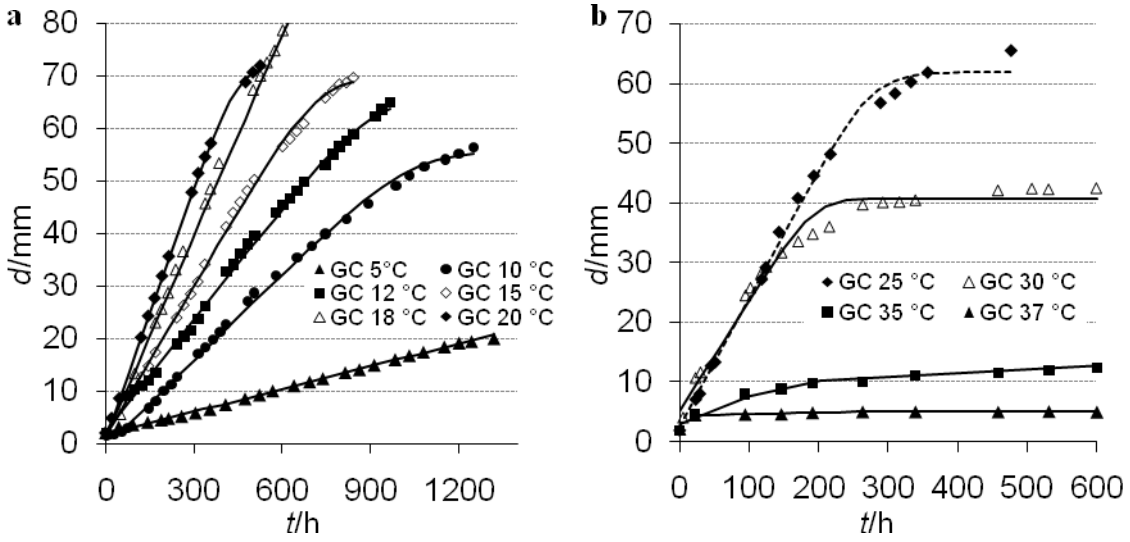

Figure 1: Colony diameter of *G. candidum* as a function of time (a) at incubation temperature ranging from 5 to 20 °C and (b) at incubation temperature ranging from 25 to 37 °C.

Table 1: Growth parameters of *G. candidum* grown on the surface of SMA agar

T [°C]	g [mm h^{-1}]	g [mm d^{-1}]	d_{end} [mm]
5	0.014	0.34	-
10	0.054	1.31	55.8
12	0.071	1.70	66.4
15	0.095	2.28	70.1
18	0.128	3.08	114.1
20	0.159	3.82	72.5
25	0.216	5.19	61.9
30	0.186	4.46	40.8
35	0.045	1.09	21.3
37	0.003	0.06	5.1

g is radial growth rate of *G. candidum* and d_{end} is final diameter of colonies

Secondary mathematical analysis

In the second part of mathematical modelling, the radial growth rate of *G. candidum* was analysed in relation to the incubation temperature. At suboptimal conditions, the effect of temperature on the radial growth rate was evaluated by Ratkowsky model (Fig. 2). Within temperature interval ranging from 5 to 25 °C the radial growth rate showed strong linear relationship to the temperature and resulting model (Eq. 9) is described with high correlation:

$$\sqrt{g} = 0.0165(T - T_{min}) + 0.111 \qquad R^2 = 0.9857 \qquad (9)$$

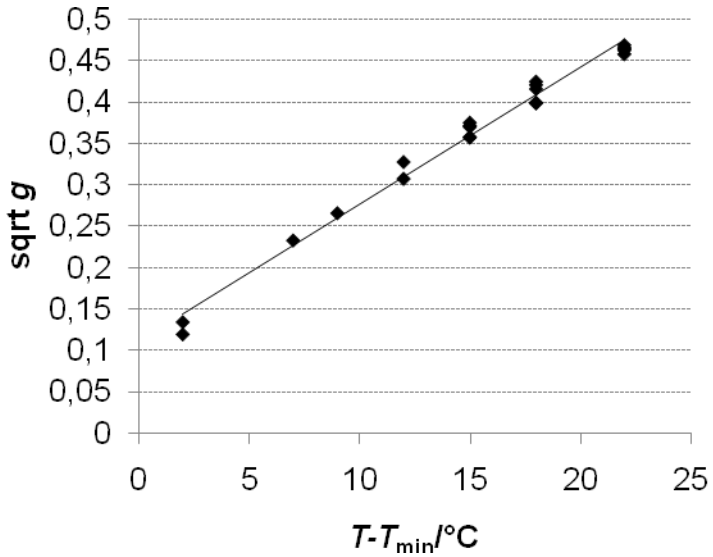

Figure 2: Ratkowsky model designed for radial growth rate of *G. candidum* on the surface of SMA agar.

Ratkowsky model of this form is able to predict the microbial growth only at suboptimal temperatures. Therefore, the calculation of optimal temperature for fungus growth is impossible from this model. On the other hand, the transformation of G-model (Gibson at al. 1994) can be used for the prediction of the entire growth response of microorganism to the incubation temperature. The temperatures ranging from 5 to 37 °C were used for modelling, and temperature over which no growth is observed was estimated to be 38 °C. The fitting of the radial growth rate data is shown in Fig. 3 and the resulting model is described with following equation:

$$\ln g = -9.2321 + 4.2351 T_w - 0.5826 T_w^2 \qquad R^2 = 0.9767 \qquad (10)$$

The model performance is demonstrated in Fig. 4. The optimum temperature of 24.8 °C for maximum growth rate of *G. candidum* was calculated using Eq. 4 and 2. This value is comparable to the optimal growth temperature of *G. candidum* reported by Boutrou and Guéguen (2005) of about 25 °C.

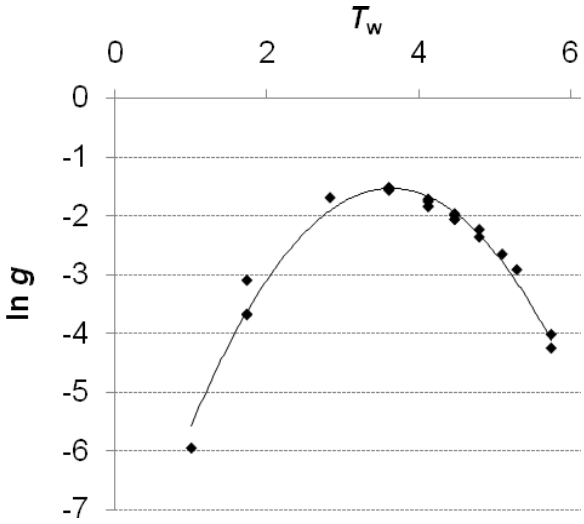

Figure 3: Parabolic fitting of natural logarithm of the radial growth rate against the transformed temperature. Symbols indicate the growth rates derived from growth curves and the line demonstrate the fitted ln g vs. T_w function (Eq. 3).

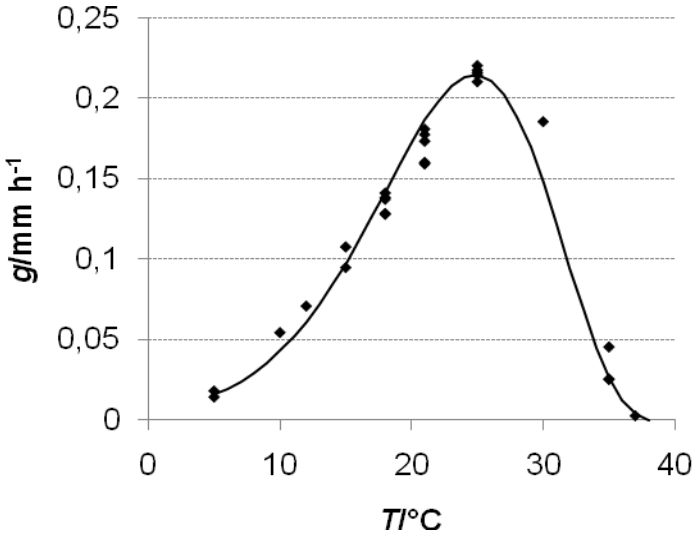

Figure 4: Plot of radial growth rate versus temperature. Symbols represent the growth rates obtained from the primary modelling and the line represents the fitted g vs. T function where g is calculated according to Eq. 3 and T according to Eq. 2.

Important parameter for the evaluation of food spoilage by fungi is also the time needed to reach the colony diameter of 3 mm (t_3), because colonies with such diameter would be visible. Like for radial growth rate also for t_3 the modelling procedure of Gibson et al. (1994) could be repeated with transformation of temperature. Resulting model is described by equation (11):

$$\ln t_3 = 10.331 - 4.2351T_w + 0.5826T_w^2 \qquad (11)$$

This model enables to calculate easily the time needed to reach 3 mm colony for any temperature. For example, at optimal temperature *G. candidum* reaches 3 mm colony diameter after almost 14 h.

Validation of secondary models

Secondary models used in this study were subjected to the validation according to Baranyi et al. (1999). Accuracy factor, bias factor, percentage discrepancy and mean square error were calculated for both, Ratkowsky model (Eq. 9) and transformed G-model (Eq. 10) and are summarized in the Tab. 2. Comparison of growth rates obtained from growth curves and growth rates predicted by used models is presented in Fig. 5.

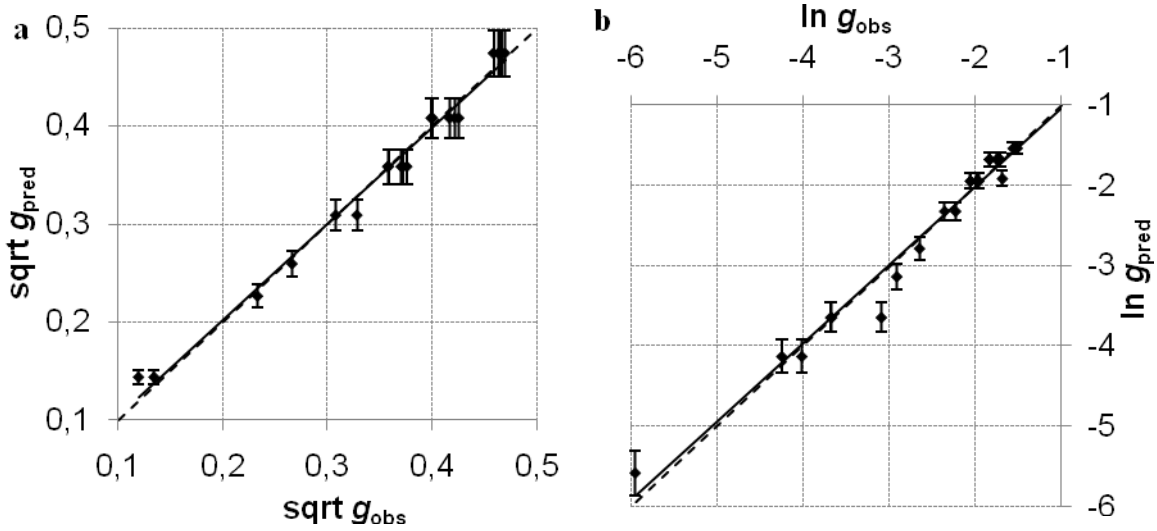

Figure 5: Comparison of observed and predicted growth rates of *G. candidum* growing on the surface of SMA agar for **(**a) Ratkowsky model and (b) transformation of G-model.

g_{obs} – observed radial growth rate, g_{pred} – predicted radial growth rate. Line of identity is dashed.

Table 2: Models performance indices as reported by Baranyi et al. (1999) for used secondary models.

Model	A_f	B_f	D_f	R^2	*MSE*
Equation 9	1.109	1.007	10.9	0.9857	$7.0x10^{-5}$
Equation 10	1.177	1.000	17.7	0.9767	0.0002

Direct comparison of observed and predicted growth rates and the validation parameters indicate good fit of the growth data on the surface of SMA agar for both models. Because there are no results concerning the secondary modelling of *G. candidum* in the literature, the performance of models used in this study was compared with the secondary models developed for other fungi. Baert et al. (2007) used Ratkowsky model in order to describe the temperature effect on the growth rate of 5 strains of *Penicillium expansum* growing on the surface of apple puree agar medium with resultant bias factors ranging from 0.91 to 1.10 and accuracy factors ranging from 1.05 to 1.19. The combined effect of temperature and water activity on the radial growth of *Botritis cinerea* was studied by Lahlali et al. (2007) who recorded accuracy and bias factors ranging from 1.070 to 1.260 and from 0.860 to 1.036, respectively. Samapundo et al. (2005) modelled the radial growth of *Fusarium verticilliodes* and *Fusarium proliferatum* growing on the maize with resulting accuracy factors ranging from 1.098 to 1.380 and bias factors ranging from 0.978 to 1.054.

Conclusion

In the present work, two models predicting radial growth of *G. candidum* were investigated. Ratkowsky model and transformation of G-model were found to be capable predictors of the growth rates of *G. candidum* in relation to the incubation temperature. Because there is a lack of quantitative data concerning *G. candidum* growth in the literature, this study contributes to the general knowledge in the field of predictive mycology. With respect to ubiquitous occurrence of the fungus, and because its ability to grow at refrigerator temperatures, there is also a need to prevent food contamination by this microorganism, especially, in products where its presence could be considered as damaging. Therefore, for practical reasons the t_3 model was designed in order to estimate the time in which the fungus growth caused spoilage of the product.

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References

Baert K, Valero A, De Meulenaer B, Samapundo S, Ahmed MM, Bo L, Debevere F, Devlieghere F (2007) International Journal of Food Microbiology 118: 139-150;

Baranyi J, Pin C, Ross T (1999) International Journal of Food Microbiology 48: 159-166;

Baranyi J, Roberts TA, McClure P (1993) Food microbiology 10: 43-59;

Boutrou R, Guéguen M (2005) International Journal of Food Microbiology 102: 1-20;

Boutrou R, Kerriou L, Gassi JY (2006) International Dairy Journal 16: 775-783;

Chapman HR, Sharpe ME (1990). In: ROBINSON RK (Ed) The microbiology of milk products, Vol 2 (pp 203-291). Elsevier Science Publishers, New York;

Cosentino S, Fadda ME, Deplano M, Mulargia AF, Palmas F (2001) International Journal of Food Microbiology 69: 53-58;

Dieuleveux V, Lemarinier S, Guéguen M (1998) International Journal of Food Microbiology 40: 177-183;

Erdogan A, Gurses M, Sert S (2003) International Journal of Food Microbiology 85: 83-85;

Fadda ME, Mossa V, Pisano MB, Deplano M, Cosentino S (2004) International Journal of Food Microbiology 95: 51-59;

Gibson AM, Baranyi J, Pitt JI, Eyles MJ, Roberts TA (1994) International Journal of Food Microbiology 23: 419-431;

Godič Torkar K, Vengušt A (2008) Food Control 19: 570-577;

Görner F, Valík Ľ (2004) Aplikovaná mikrobiológia požívatín. Malé centrum, Bratislava;

Hayaloglu AA, Kirbag S (2007) International Journal of Food Microbiology 115: 376-380;

Kocková-Kratochvílová A (1990) Taxonómia kvasiniek a kvasinkovitých mikroorganizmov. Alfa, Bratislava;

Lahlali R, Serrhini MN, Friel D, Jijakli MH (2007) International Journal of Food Microbiology 114: 1-9;

Laurenčík M, Sulo P, Sláviková E, Piecková E, Seman M, Ebringer L (2008) International Journal of Food Microbiology 127: 176-179;

Lecocq J, Gueguen M (1994) Journal of Dairy Science 77: 2890-2899;

Marcellino N, Beuvier E, Grappin R, Guéguen M, Benson DR (2001) Applied and Environmental Microbiology 67: 4752-4759;

Nielsen MS, Frisvad JC, Nielsen PV (1998) International Journal of Food Microbiology 42: 91-99;

Plaza P, Usall J, Teixidó N, Viñas I (2004) International Journal of Food Microbiology 90: 75-82;

Pottier I, Gente S, Vernoux JP, Guéguen M (2008) International Journal of Food Microbiology 126: 327-332;

Samapundo S, Devlieghere F, De Meulenaer B, Geeraerd AH, Van Impe JF, Debevere JM (2005) International Journal of Food Microbiology 105: 35-52;

Tornadijo ME, Fresno JM, Sarmiento RM, Carballo J (1998) Le Lait 78: 647-659;

Valík Ľ, Piecková E (2001) International Journal of Food Microbiology 63: 11-17;

van den Tempel T, Nielsen MS (2000) International Journal of Food Microbiology 57: 193-199;

Varnam AH, Sutherland JP (1994) Milk and milk products: Technology, chemistry and microbiology. Chapman and Hall, London;

Wouters JTM, Ayad EHE, Hugenholtz J, Smit G (2002) International Dairy Journal 12: 91-109.