Faculty of Food Science

PREDICTIVE MODELLING OF THE GROWTH OF POTENTIALLY TOXIN PRODUCING MOULDS AS A FUNCTION OF TEMPERATURE AND WATER ACTIVITY

Ph. D. THESIS

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1. BACKGROUND

After harvest, the so-called postharvest losses caused largely by microbial, mainly mould deterioration, are responsible not only for increasing the economic costs, but they also present food safety risks. Mould growth is a hazard that needs attention in all stages of food production - primary production (agriculture), storage, processing and marketing. For the food industry, it is essential that raw materials are free from mould and mycotoxin contamination.

Beside to the quantitative demand for food production, food quality is also a point to take into account. The demand for high quality, healthy food is a strategic question all over the world.

The extreme weather, one of the consequences of the global climate change, have unfavourable impacts on both food security and food safety, for example due to the more and more common occurrence of toxigenic moulds. The increasing frequency and duration of heat-wave periods cause food safety concerns via the growth and toxin formation of the thermophilic black aspergilli. It is of great importance to study the effects of temperature and water activity, which are the most important environmental factors, on the growth of potentially toxigenic moulds.

For these reasons, it is of key importance to study/predict mould growth under different eco-physiological conditions. Many data, from a wide range of conditions are required to develop mathematical models that will be suitable to describe microbiological risk and to prevent mould growth. It is therefore timely to study the effect of environmental conditions on the growth of moulds.

2. AIMS

The aim of the work was to model the growth of moulds as affected by the most important environmental factors, temperature and water activity.

The objectives were to

- collect relevant data on the growth of potentially toxigenic moulds;
- investigate the combined effect of temperature and water activity on the growth of the selected moulds (*Aspergillus niger* and *Penicillium expansum*);
- select/create a mathematical model in order to describe the growth of *Aspergillus niger* as a function of temperature and water activity;
- develop a ComBase-compatible Mycology Database.

3. MATERIALS AND METHODS

3.1 Fungal strains

Aspergillus niger F.00770 and *Penicillium expansum* F.00811 were obtained from the Hungarian National Collection of Agricultural and Industrial Microorganisms, Corvinus University of Budapest, Hungary.

3.2 Media

- Malt Extract Agar (Merck)
- Grape medium (juices of 4 grape varieties freshly pressed in the lab fortified with agar)
- Apple medium (commercial 100% apple juice fortified with agar)

The accuracy of the microbial response derived from parallel measurements and from repeating the experiments was investigated at 20, 25, 30 and 35°C and water activities (a_w) 0.90; 0.92; 0.94; 0.96; 0.98 and 0.99. The diameters (mm) of the colonies were measured three times a day, until the plates were completely colonized or up to maximum 36 days.

3.3 Methods

The water activity was set by the addition of known amounts of glycerol (*P. expansum*) and NaCl (*A. niger*) to 0.90; 0.92; 0.94; 0.96; 0.98 and 0.99. The water activity of the media was determined with a NOVASINA LabMaster.a_w Instrument (VITALIS, Hungary), which was calibrated against saturated salt solutions in a range of known water activities. The a_w of the inoculated media was measured after 36 days, at each temperature, and no change was detected.

Regression analysis and ANOVA (macros of Microsoft Excel®) were used as statistical methods.

Colony diameter versus time data were fitted by the model of Baranyi and Roberts, using an in-house Excel Add-in package "DMFit" (http://www.combase.cc/index.php/en/downloads/file/53-dmfit-30) in order to determine the growth rate.

4. RESULTS

The results demonstrated that *Aspergillus niger* F.00770 needs significantly higher temperature (30-35°C at a_w 0.98) to grow, than *Penicillium expansum*. The maximum growth of *P. expansum* F.00811 was detected at 25°C and a_w 0.98. These results were in good agreement with data from literature.

The sources of the prediction error were analysed. The experimental variability was quantified by the relative accuracy of measuring the colony growth rate, which error was estimated as ca. 5% when colonies grew in parallel. The environmental variability was estimated by the relative accuracy (17%) of the averages of repeated growth experiments on the colony growth rates. This accuracy for a secondary model can be achieved only if it fits the grand-means of the repeats perfectly. This is less and less achievable as the studied region of the environmental variables increases. We can say that, in the region we focus on, an independent single measurement of the growth rate, which is carried out similarly as we did, will deviate from our prediction by ca. 20%.

The quadratic model used in the error analysis is only valid in a local environment, therefore another secondary model, stemming from the so-called cardinal values model has been developed, which is more suitable for prediction also at points close to the boundaries of growth. The combined model was validated by independent data. Two from 24 data points did not fall into the confidence band of the combined model, which represents well the accuracy of the 95% confidence interval.

Model food substrates: grape juice (prepared from 4 different grape cultivars, freshly pressed in the lab) and apple juice (commercial, 100% juice) media were inoculated with conidia of *A. niger* and *P. expansum*, respectively, to get more information on mould growth. The calculated growth rates from the measured growth data of *A. niger* on grape juice media followed the same tendency as that of the combined model even with bias. *P. expansum* grew faster in most cases on malt agar media than on the apple juice medium. The reason for this observation could be that apple juice is poorer in nutrients than the malt agar medium.

A new database for ComBase has been developed focusing on mould growth under various environmental conditions. The objective is to provide an electronic repository that can help to increase the translational efficiency of research in predictive microbiology and its application. Data have been, and is continuously going to be, collected from research establishments and publications. The output will serve as an invaluable tool for research and education purposes, for food microbiologists, industrialists, and regulatory offices.

The results demonstrated that *Aspergillus niger* has significantly higher temperature requirement than *Penicillium expansum*. Therefore, the projected increase of environmental temperature, particularly the increasing frequency and duration of heat-wave periods may cause an elevated risk of compromising mycological food safety by stimulating the growth of the presently common *Penicillium* species and creating better opportunity for growth and toxin formation of the rather thermophilic black aspergilli by contaminating more intensively our fresh produce in the field and causing problems also during storage.

These results contribute to the possible prediction of mould growth as a function of temperature and water activity. They can be utilized in practice and in risk assessment procedures as well.

5. NEW SCIENTIFIC RESULTS

1. Error analysis in predictive modelling of growth of *Aspergillus niger* was described. The variance of replicates was used to quantify the experimental variability, which was shown to be ca. 5%. The environmental variability (17%) was quantified by the variance of the repeats. The idea is analogous to the „within group" and „between groups" variability concepts of ANOVA procedures. An independent single measurement of the growth rate will deviate from the prediction by ca. 20%.

2. A new model was used to predict the growth of *Aspergillus niger*. It was based on a combination of the so-called cardinal values model (minimum, optimum and maximum values) for the temperature, and a quadratic model for a rescaled version of the water activity. By the use of the resultant combined model, its validity domain can be extended to the limits of the mould growth, contrary to the quadratic response surface used for error analysis, which can be used for interpolation only. By varying the leading coefficient, the quadratic response surface will be asymmetric, which reflects the trend of the data better.

3. The applicability of the combined model was investigated on the growth of moulds on natural (pressed grape juice) medium, too. The calculated growth rates followed the same tendency as that of the combined model. Under unfavourable environmental conditions (minimum conditions for growth) grape juice media supports the growth of *A. niger* better than the malt medium. Apple juice medium, however, supports less the growth of *P. expansum*, even under optimal environmental conditions. It confirms the need for validating the predictions under natural conditions.

4. The ComBase predictive microbiology database was expanded with a new set of data, focusing on the growth of moulds under different environmental conditions. The mycological database of ComBase started with the data from this study.

6. PUBLICATIONS

Scientific papers with IF:

Csernus, O., Andrássy, É., Bata-Vidács, I., Beczner, J., Farkas, J. (2011): *Penicillium expansum* és *Aspergillus niger* növekedési hőmérséklet- és vízaktivitás- függésének vizsgálata, különös tekintettel a klímaváltozásra. *Élelmiszervizsgálati Közlemények,* 57 (4) 209-218 (IF: 0,040)

Csernus, O., Bata-Vidács, I., Farkas, J., Beczner, J. (2013): Effects of environmental temperature and water activity on growth of *Aspergillus niger* and *Penicillium expansum*. *Acta Alimentaria*, 42 (4) 640-648 (IF: 0,43)

Baranyi, J., **Csernus, O.,** Beczner, J. (2014): Error analysis in predictive modelling demonstrated on mould data. *International Journal of Food Microbiology*, 170 78-82 (IF: 3,425)

Paper in Hungarian:

Csernus, O. (2012): Penészgomba szaporodás előrejelzése – Mikológiai adatbázis. *Konzervújság*, LVIV. Évf. (3-4.) 52-53

Conference summaries:

Csernus, O., Baranyi, J. (2011): Data mining for training in food microbiology. NetSci-2011 Network Science Applications to Food Security and Safety. Book of abstracts. p. 8.

Csernus, O., Baranyi, J., Beczner, J., Farkas, J. (2011): Knowledge mining from microbiological data – application of network science in food microbiology. *Acta Microbiologica et Immunologica Hugarica* Vol. 58, Supplement. p. 133.

Csernus, O., Baranyi, J. (2012): Mycology database on prediction of mould's growth. 5th Hungarian Mycological Conference. Book of abstracts Vol. 51. No.1. pp. 54-56.

Csernus, O., Dalmadi, I., Bata-Vidács, I., Andrássy, É., Beczner, J., Farkas, J. (2013): The use of electronic nose for rapid estimation of mould contamination of fruit products. 4th MoniQA International Conference, 26 February – 1 March 2013, Budapest. Book of abstracts p. 105.

Csernus, O., Bata-Vidács, I., Andrássy, É., Beczner, J., Farkas, J. (2013): Effect of environmental temperature on growth of *Penicillium expansum* and *Aspergillus niger*, with special reference to the climate change. 4th MoniQA International Conference, 26 February – 1 March 2013, Budapest. Book of abstracts p. 106.

Csernus, O., Baranyi, J. (2013): Predictive Mycology. 4th MoniQA International Conference 26 February – 1 March 2013, Budapest. Young Scientist Forum. Book of abstracts pp. 164-165.