Journal of Industrial Microbiology, 12 (1993) 324–329

Published by The Macmillan Press Ltd

A decision support system for prediction of microbial spoilage in foods

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(Received 13 October 1992)

Key words: Modeling; Bacterial growth; Food quality; DSS

SUMMARY

A method is developed to combine qualitative and quantitative information for the prediction of growth of microorganisms in foods. pH, water activity, temperature and oxygen availability of foods are coupled to growth characteristics of microorganisms. For that purpose, a database with characteristics of foods and a database of kinetic parameters of microorganisms are built. The first database has a tree structure, based on physical similarity of food products. This structure makes it possible to estimate information about a food product which is not listed by comparison with similar products at the same level of the tree or the level above. A method is developed to make an estimation of the microbial growth kinetics on the basis of models. This is done by introducing a growth factor, which can be calculated on the basis of readily available data from literature. Finally, qualitative knowledge is added. Since any bit of information can be changed, the system will give better predictions when more and more accurate information is added.

INTRODUCTION

Food quality can be defined as the sum of the characteristics of a food that determines the satisfaction of the consumer and compliance to legal standards. Quality loss can be a result of microbial growth, or chemical, physical, or enzymatic reactions. There are numerous factors that can influence this quality loss, like the composition of the product, and processing and storage conditions. Prediction of the kinetics of possible quality loss is important for the following reasons. The food market deals in most cases with saturation, therefore quality becomes more important than quantity. There is a rapid new product development, and large product diversification. During product development there is often not enough time for shelf-life testing. Especially microbial risks are larger, since many products are stored chilled (no sterilization), and often less salt, acid and preservatives are added.

DATABASES

The organism database

A database is built for microorganisms that contains the name of the organism and the growth ranges of the physical variables: oxygen requirement, the minimum and maximum pH, a_w, and temperature (Fig. 1). Additionally, the optimum growth rate and the optimum values of pH and temperature are stored, to be used in kinetic models. Furthermore the Gram-staining, type, and spore-forming abilities are stored, to be used for further selection procedures (qualitative reasoning) or future use. In total 20 genera of Gram-negative bacteria, 19 genera of Gram-positive bacteria, 10 yeast species, and 9 mold genera are incorporated in the organism database at present. If the optimum growth rate of the organism is not known, for bacteria 1 h^{-1}, for yeasts 0.5 h^{-1}, and for molds 0.1 h^{-1} is assumed.

This database already gives useful information, however it is useful to couple it with characteristics of foods.

The food database

A database is built that contains the physical variables of different foods. As may be expected, not all the physical variables of all different foods are known. Therefore the database is structured in such a way, that the products are sorted with respect to their physical properties so that foods that are grouped together are closely related. The classification system proposed by Jowitt [1], based on the physical properties of foods is therefore used. In such a way missing information, even when a product is absent, can be substituted with knowledge from comparable products.

Selection of organisms that can grow on a particular product

As soon as the physical variables of a product are known, a matching is carried out with the organisms database (Fig. 2). All the organisms that can grow on that product are found by 'pattern matching' of the physical variables. The procedure results in a list of organisms which can grow in a certain product with particular physical variables.

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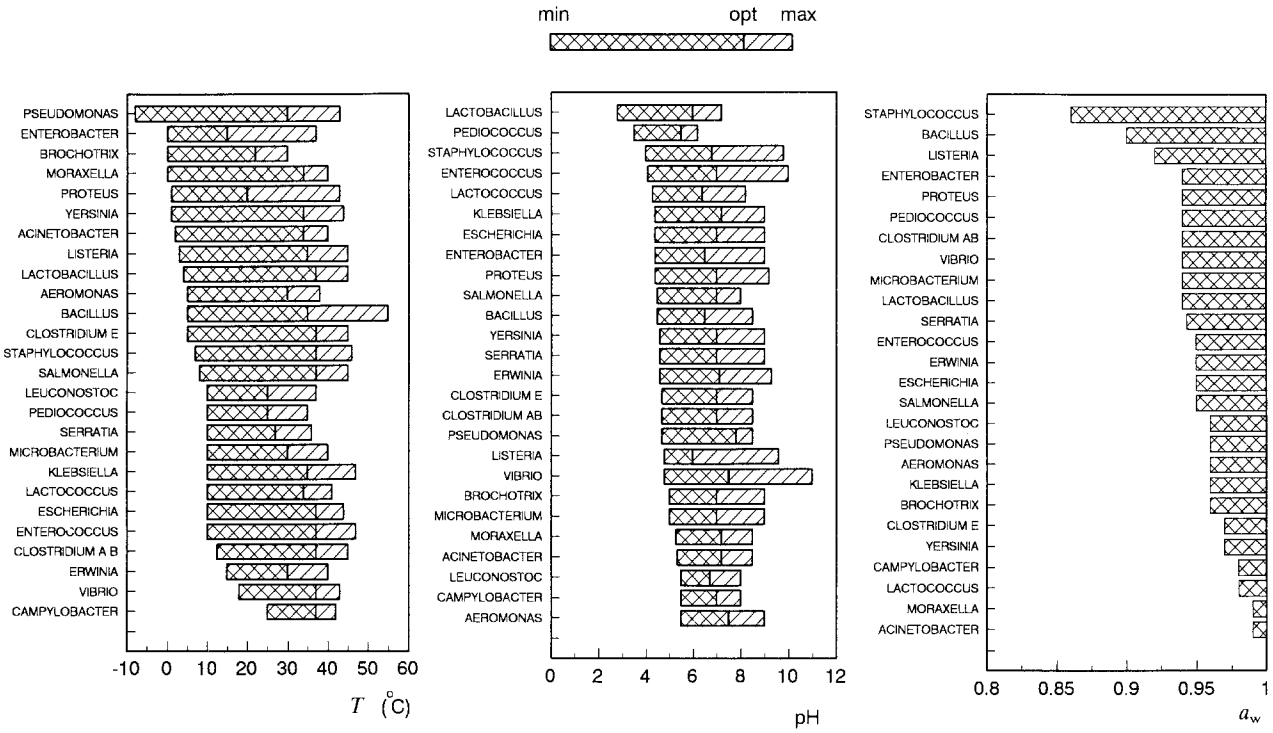

Fig. 1. Microorganism database.

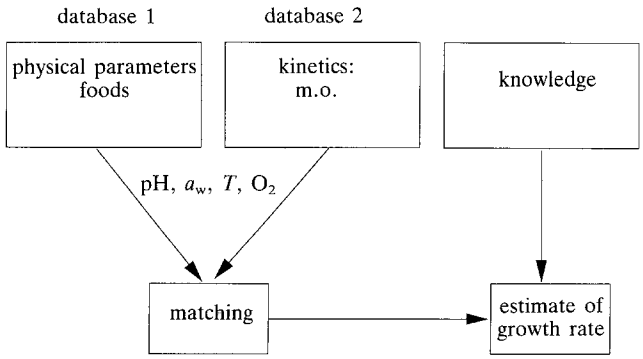

Fig. 2. Structure of the system [5].

If a certain organism can grow on a product it is useful to know how fast it will grow. Therefore kinetic models must be developed.

KINETIC MODELS

Growth rates of 38 growth curves of a *Lactobacillus plantarum* strain at different temperatures were estimated [4]. These data can be described by different types of models. Often the logarithm of the growth rate is modeled using a polynomial function:

$$\ln(\mu) = a + bT + cT^2 \tag{1}$$

These models have the advantage that they are linear and very straightforward. Furthermore, other variables can be very easily added, for instance:

$$\ln(\mu) = a + b \cdot T + c \cdot T^2 + d \cdot pH + e \cdot pH^2 + f \cdot a_w + g \cdot a_w^2 + h \cdot T \cdot pH + i \cdot T \cdot a_w + j \cdot pH \cdot a_w \tag{2}$$

The quadratic polynomial (Eqn 1) model can describe the effect of temperature, however at higher temperatures, discrepancies can be seen since the model is symmetrical, and the data show an asymmetrical shape (Fig. 3). This can be seen especially when the growth rate is plotted (instead of the logarithm of the growth rate).

The model of Ratkowsky [3] is able to describe the effect of temperature on growth rate over the whole temperature range (Fig. 3) and is simple, therefore this model is chosen for the kinetic models.

To estimate the growth rate of organisms at suboptimum conditions for T, a_w, and pH, models have to be used. The growth rate can be estimated using models relating growth at the actual value of a variable to the optimum value and the limits. Each variable that is not at the optimum value can reduce the growth rate. Therefore a method to combine these effects must be established. This is done by introducing a growth factor:

$$\gamma = \frac{\mu}{\mu_{opt}} \tag{3}$$

where μ = the actual growth rate (h^{-1}), μ_{opt} = the growth

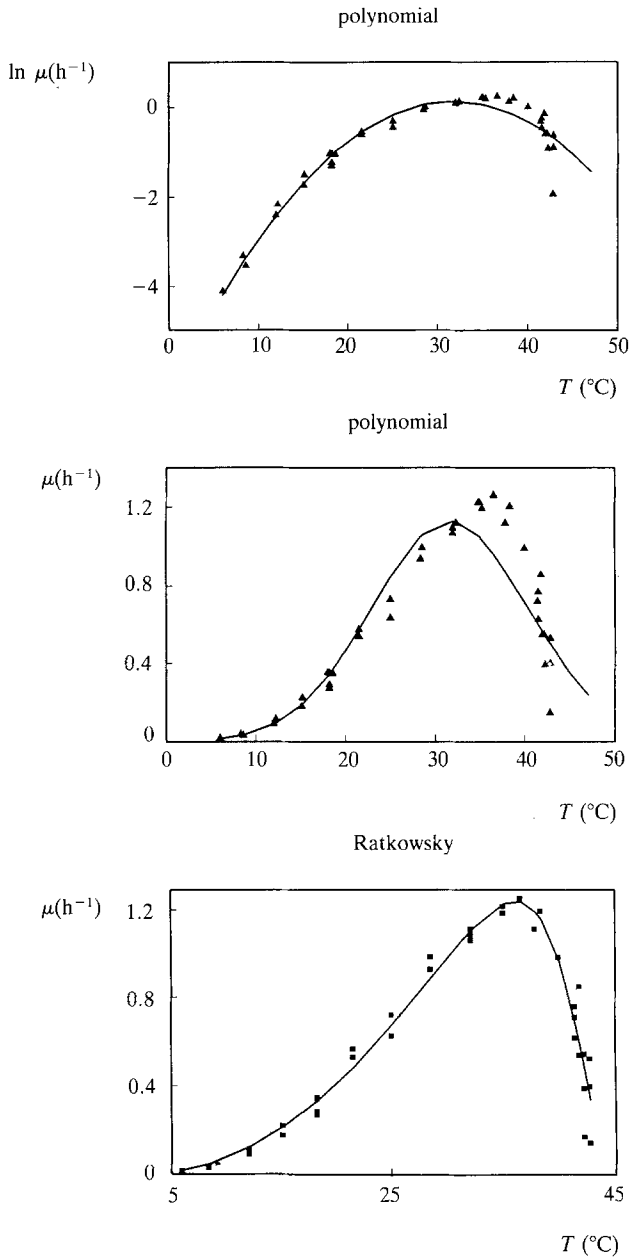

Fig. 3. Comparison growth rate versus temperature.

rate (h^{-1}) at optimum conditions, and γ = the actual growth factor.

This growth factor is equal to 1 at optimum conditions and between 0 and 1 for all other conditions. It is assumed that the growth factor can be calculated by multiplying all $\gamma(\chi)$ values, with $\gamma(\chi)$ defined for each of the variables separately, independent of the value of the other variables:

$$\gamma = \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) \cdot \gamma(O_2) \tag{4}$$

If all variables are at optimum conditions the growth rate is equal to μ_{opt}. If one of the variables is below the minimum or above the maximum value, this results in one of the γ's being zero, resulting in a growth rate of zero.

Each $\gamma(\chi)$ factor can be determined from the database data, in combination with a model for that variable. In the microorganism database the minimum, optimum and maximum temperature for growth of different organisms can be found. If these data are known the parameters of the Ratkowsky equation [3] can be calculated:

$$\mu = (b(T-T_{min})\{1 - \exp[c(T-T_{max})]\})^2$$
$$T_{min} \leq T \leq T_{max} \tag{5}$$

where b, c, T_{min}, T_{max} = Ratkowsky parameters and T = actual temperature (°C).

If c is known, the growth factor $\gamma(T)$ for each temperature value can be evaluated with:

$$\gamma(T) = \frac{\mu}{\mu_{opt}} = \left(\frac{(T-T_{min})\{1-\exp[c(T-T_{max})]\}}{(T_{opt}-T_{min})\{1-\exp[c(T_{opt}-T_{max})]\}}\right)^2 \tag{6}$$

The value of c can be calculated from the known T_{min}, T_{max}, and T_{opt}. The derivative of Eqn 5 must be zero at $T = T_{opt}$. This results in:

$$1 - (cT_{opt} - cT_{min} + 1) \cdot \exp[c(T_{opt}-T_{max})] = 0 \tag{7}$$

c can be calculated iteratively from this equation, to be used in Eqn 6.

No good models are available for pH over the whole range. Therefore, the same formula from Ratkowsky [3] is used, only pH is substituted for T.

McMeekin et al. [2] showed that the relation of growth rate and a_w is linear at suboptimum water activity levels. For the water activity therefore a linear relation is assumed:

$$\gamma(a_w) = \frac{a_w - a_{w,min}}{1 - a_{w,min}}$$
$$a_w \geq a_{w,min} \tag{8}$$

where $a_{w,min}$ = minimum water activity, and a_w = actual water activity.

Oxygen availability is used as a selection parameter ($\gamma(O_2)$ = 0 or 1, Table 1). For oxygen availability this segmentation model is used, since for most microorganisms the growth kinetics as a function of the oxygen availability are not known. The same is true for the oxygen concentration in products. If models and model parameters are known this can be altered, since the segmentation model as shown in Table 1 is a very rigorous one.

The combination model selected and used here is not yet thoroughly validated. It can be used however, to make kinetic predictions, although the numerical value is indeed a prediction only. Yet, a good estimate is made about the growth rate. Whenever more knowledge is present for models describing the effect of the variables used here, these models can be incorporated. It should be noted that there are no interaction effects assumed between T, a_w, pH, and O_2.

TABLE 1

The effect of the availability of oxygen on $\gamma(O_2)$ [5]

	Organism: aerobic	Organism: facultative anaerobic	Organism: anaerobic
Product with oxygen	1	1	0
Product with very little oxygen	0	1	0
Product with no oxygen	0	1	1

There are many more variables determining the growth rate of microorganisms, such as preservatives (sorbic and benzoic acids, alcohol, nitrite). These compounds will often be present in specific products, such as alcohol in alcoholic beverages, and nitrite in meat products. The effect of these compounds can be incorporated by applying knowledge rules. This method is simpler than the use of kinetic models since at present models and model parameters are not well established.

Only if all knowledge is present can exact predictions be made. This is an impossible situation, therefore every result will always be an approximation.

Addition of qualitative reasoning

Knowledge can be added to the system (Fig. 2) to decrease the number of possible organisms that can cause problems. Before these rules are applied, the user is asked if this rule is applicable, since they can be too stringent sometimes.

RESULTS AND DISCUSSION

The program was developed using TURBO-Pascal 5.0 (Borland). The system is started entering the name of a food. The program then searches database 1 for the name of that food, or foods with a very similar name. Within this list of names a final selection can be made for the food of interest. The physical variables of this product are displayed when present in the database. If the physical variables of the product are not known, comparable products (if any) are given. An estimation of the physical variables can be obtained by a selection from this list.

With the physical variables of the food, a matching is carried out with the organisms database. The organisms that can grow on that product considering the physical variables (pH, T, a_w, and oxygen availability) are determined by 'pattern matching' with the growth ranges of the organisms. This results in a list of microorganisms that can grow on the food together with the growth factor (Eqn 4). Now rules can be applied to diminish the number of organisms in the list or to improve the value of the predictions.

Output

Two lists of possible spoilage organisms are generated. The first list contains the possible spoilage organisms and pathogens, based on physical variables. The second list contains the knowledge rules that are applied, and the list of organisms that are likely to spoil the product, after the knowledge rules are applied. As output, not only the final list should be considered, the first list also contains valuable information. For example, if a pasteurization is carried out, the final list will give no thermosensitive organisms. However, if the product is contaminated after the pasteurization the thermosensitive organisms can be of interest.

Possible expansion of the system

A large amount of knowledge is present on quality loss processes in foods, depending on composition, process variables, and kinetics. It could be useful to develop a system in which this knowledge is combined. The system can be expanded by including chemical, enzymatic, and physical spoilage in the same manner as described. In this way, effects of a large number of changes in the product or process can be evaluated. This can be done for instance for the addition of onions to a salad dressing (microbial, enzymatic, physical). The effect of chemical and microbial spoilage when a heat treatment is carried out at a higher temperature can be evaluated, as can the effect of storage in a modified atmosphere (if the effects of gases are known). For product development, the possible spoilage reactions, the order of magnitude of these reactions, the approximate shelf-life, and distribution temperature can be evaluated. By using more or less complicated models and kinetic parameters, predictions can be made of these deterioration reactions.

EXAMPLE FOR SAUSAGE

Selection of organisms

During storage of a sausage a temperature of 7 °C can be achieved. The atmosphere is aerobic. In the components database the following data can be found for sausage: pH = 4; a_w = 0.98. In the organism database the data of all organisms are examined. For instance, for *Lactobacillus* the data are given in Table 2. Combining this information results in the deduction that *Lactobacillus* can grow in sausage of pH = 4. If this 'pattern matching' is carried out for all organisms the system comes up with 2 bacteria, 6 molds and 9 yeast species.

TABLE 2

An example of the information stored in the organism database

Genus				*Lactobacillus*		
Species				*plantarum*		
Oxygen necessity				fac. aerobic		
Type (bacterium, yeast, mold)				bacterium		
Gram-stain (only for bacteria)				positive		
Spore forming				no		
T	min	max	opt	4	45	37
pH	min	max	opt	2.8	7.2	6
a_w	min	max	opt ≈ max	0.94	1.000	
optimum growth rate (h^{-1})				1.4		

Kinetic estimation

For all organisms that can grow on sausage, the growth factor will be calculated (Eqn 4). This will be done as an example for *Lactobacillus*.

For the temperature Eqn 7 can be used, using the kinetic data of *Lactobacillus* (Table 2). This function is given in Fig. 4. This equation can be solved iteratively and results in $c = 0.30$. With Eqn 6, the $\gamma(T)$ can be calculated at every temperature (Fig. 5).

For the pH also, Eqn 7 can be used resulting in $c = 1.43$. With Eqn 6, the γ(pH) can be calculated. For the a_w, Eqn 8 can be used.

For sausage the following growth factors are calculated:

for $T = 7$, $\gamma(T) = 0.010$;
for pH = 4, $\gamma(pH) = 0.20$; and
for $a_w = 0.98$, $\gamma(a_w) = 0.67$.

$$\gamma = \gamma(T) \cdot \gamma(pH) \cdot \gamma(a_w) = 0.00137 \tag{9}$$

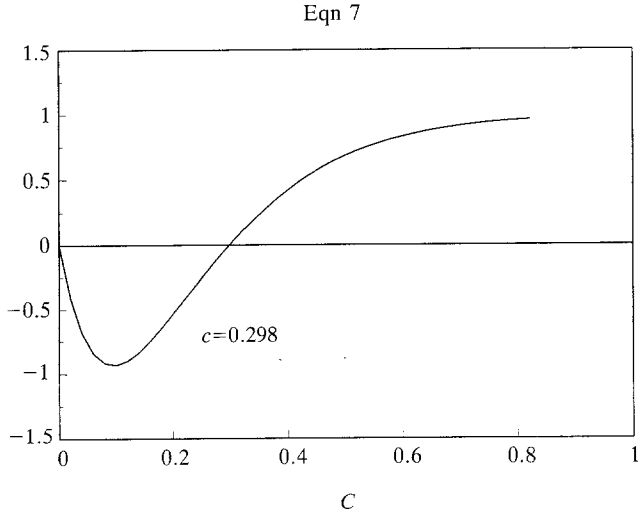

Fig. 4. Eqn 7 with data for *Lactobacillus* (T_{min}, T_{opt}, T_{max}).

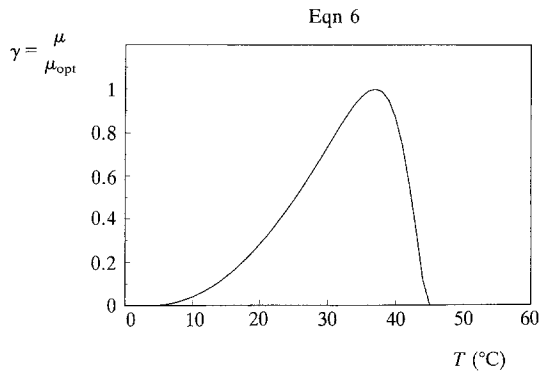

Fig. 5. Eqn 6 with data for *Lactobacillus* (c, T_{min}, T_{opt}, T_{max}).

$\mu_{opt} = 1.4\ h^{-1}$.

Now the growth rate can be estimated with Eqn 3:

$$\mu = \gamma \cdot \mu_{opt} = 0.0019\ h^{-1}. \tag{10}$$

If the product is initially contaminated with 10^3 organisms g^{-1} and if the maximum tolerated number of *Lactobacillus* is 10^6 organisms g^{-1}, the shelf-life can be estimated:

$$\Theta = \frac{\ln\left(\frac{N_t}{N_o}\right)}{\mu} = \frac{\ln(1000)}{0.0019} = 3636\ h = 151\ days \tag{11}$$

Addition of qualitative reasoning

In the reasoning the knowledge that bacteria will grow much faster than yeasts and molds can be used. An estimation of the growth rate can be made on the basis of models, describing the effect of the physical variables on the growth rate. In the example given above, the conditions in sausage are not optimum for *Lactobacillus*, therefore the growth

rate will be smaller than the optimum growth rate. It is assumed that the fastest growing organisms will cause problems (10% rule). If this knowledge is used the system comes up with only *Lactobacillus*. The estimate of the shelf-life based on growth of *Lactobacillus* is 150 days. The knowledge rule that bacteria will grow much faster than the yeasts and the molds should be reconsidered. In such a long shelf-life yeasts and molds can be important spoilers.

Change in product characteristics

If we now change the pH of the sausage from 4 to 5, and make a further prediction, there are 9 yeasts, 6 molds, and 10 bacteria that can grow on the product. If we assume that the bacteria grow faster than the other organisms, and reject the organisms that grow 10 times as slow as the fastest grower (10% rule), the main spoilers are predicted to be *Enterobacter* and *Proteus* (Table 3), and the shelf-life becomes 3 days. It can be noted that the prediction of the main spoiler changes from *Lactobacillus* to *Enterobacter*, the number of bacteria that are capable of growing increases dramatically and the shelf-life is predicted to decrease dramatically for the product at pH 5. The assumption that the bacteria will grow much faster than the yeasts and molds will be valid. For a shelf-life of 3 days yeasts and molds are often not important.

The predictions of the system should be considered as an estimation of the order of magnitude.

TABLE 3

Microorganisms that are predicted to cause spoilage in sausage of pH 5 stored at 7 °C

Species	Growth rate (h^{-1})
Enterobacter	0.101
Proteus	0.031
(*Lactobacillus*	0.0060 < 10%)

CONCLUSIONS

A system is developed which shows a promising potential for product development and shelf-life prediction. Quantitative and qualitative information, and predictive models can be combined to predict possible spoilage reactions, with an estimate of their kinetics, on the basis of models. To that purpose a database is built and filled with physical variables of foods, as is a database with organisms with their growth limits for the same physical variables. A combined model is built to be able to make a kinetic estimation, on the basis of the data in the databases. Furthermore a knowledge base is built which can be used to add qualitative information concerning products and microorganisms. The system combines all this information. Since it is impossible to collect quantitative data for all possible deterioration reactions on different products, a prediction is made on the basis of the data and knowledge collected in the system until now.

The program can help to determine possible spoilage organisms. It can estimate the change in growth rates of organisms, when the physical properties are changed. It should be noted that the program does not give an exact, complete list of all possible spoilage organisms, since it is based on limited information. The more knowledge is combined and added to the program, the better the predictions will be.

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