Predictive Microbiology: Targeted Control of Fermentation Processes

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Summary

In the production of dry sausage it is important to adjust the ripening conditions precisely to the selected starter culture, if optimum acidification performance has to be achieved. However, the effect of ripening parameters are not fully explained yet, and so far is has been impossible to achieve targeted control during the fermentation process. Therefore, a new concept of determining the acidification performance of starter cultures in dry sausage was developed.

It is distinguished by chosen experimental conditions, which correspond largely to the conditions present during dry sausage ripening and thus provide results, that can be applied under practical conditions. The combined influence of temperature and water activity as ripening factors on the lag-phase and acidification capacity of different strains of *Lactobacillus* and *Pediococcus* was investigated.

By applying the modified linear Arrhenius model of DAVEY on the data it was possible to predict the combined effects of temperature and water activity on the acidification performance and the lag-phase. The model is easy to fit to data by using linear least squares regression procedure of nearly every spreadsheet. Therefore, the acidification performance during ripening can be predicted and influenced in a targeted way by means of a simple mathematical model.

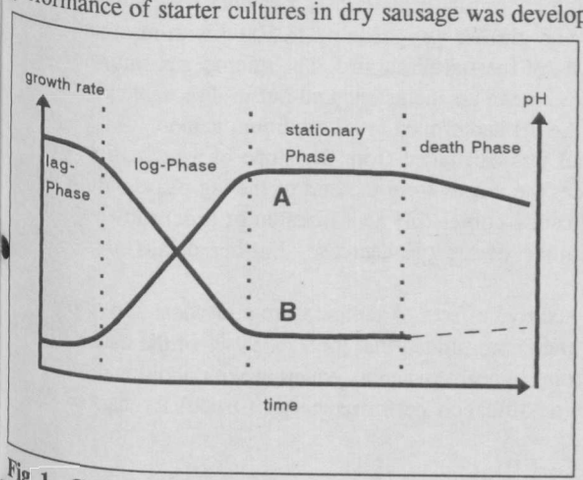

Fig 1. Growth rate (A) and acidification rate (B) by a *Lactobacillus* starter culture (schematically).

Introduction

Predictive microbiology focusses on investigations of models to predict the growth and death rates of food spoiling microorganisms or toxine production under variable environmental parameters [LEISTNER, 1990]. Based on various multivariable experiments, mathematical models have been developed to predict shelflife and hygiene of food products if several parameters are influencing the microbiological growth at the same time, as it is present for foods. Predictive microbiology so far investigated mainly the growth behavior of food spoiling strains like *Salmonella, Listeria* and *Staphylococcus aureus*.

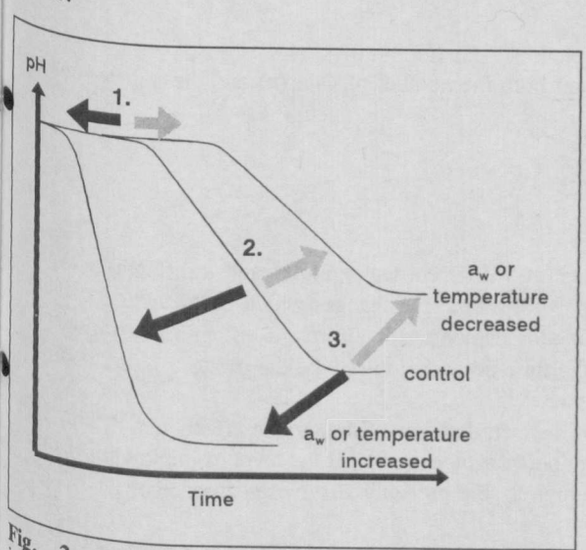

Fig. 2. Acidification kinetics of starter cultures influenced by temperature and water activity.

Mathematical models have been developed to describe either the growth of microorganisms or the inactivation regimes (e.g. botulinum cook). These principles can not only predict growth rates but are also suitable to predict the growth of starter cultures. This is of particular interest, as during fermentations the physiological properties (ability to produce lactic acid) of the starter can be measured instead of the growth rate.

Dry sausage ripening serves as a good example to varify the application of the above model on a food product. During the ripening process starter cultures metabolize glucose to lactic acid. Besides many other parameters the acidification course strongly depends on temperature and water activity. It is therefore important to adjust the ripening conditions precisely to the needs of the starter culture to optimize the acidification performance (drop in pH/h). Here it is not only important to know the lag phase and the growth rate, it is essential to predict the influences on the metabolism (lactic acid production). The principles of predictive microbiology shall be extended to predict acidification performance.

Fig. 1 explains schematically the relationship between growth rate and acidification, as it can be expected with a *Lactobacillus* starter culture. Both shapes of the curves are similar but inverse and follow the same mathematical laws. Therefore it is possible to also determine the log and the lag phase through pH measurements. Fig. 2 shows the general regime to be expected theoretically if temperature and a_w are altered. The arrows point out three different effects. Primarily the lag phase is affected, secondly the acidification performance and thirdly the extend of the pH drop is changed. These relationships are common knowledge but have up to now not been quantified, especially if two influencing parameters (temperature *and* a_w) are acting at the same time. Many methods of activity determination don't provide these informations, as they are usually carried out under quite different experimental conditions (e.g. substrate) which are not comparable to dry sausage ripening conditions.

Material and Methods

To create a test which copies best the conditions during practical dry sausage ripening a model mixture of minced meat has been prepared. The batches with different a_w have been inoculated with $3 \cdot 10^7$ CFU/g mixture starter culture. *Lactobacillus curvatus 2, Lactobacillus curvatus 3, Pediococcus pentosaceus* and *Pediococcus acidilactici* (Gewürzmüller, Stuttgart, Germany) have been used.

Adjustment of different a_w values: 2,6% nitrite curing salt was added to each batch (except for a_w=0.993). Different a_w values were adjusted by adding increasing amounts of common salt. Glucose as a substrate for fermentation was added to a level of 1% to not limit the fermentation process. All starter cultures metabolized glucose. The temperature influence was tested in the range of 10, 15, 20, 25 and 30 °C.

Preparation of the batches: The chilled meat was minced all at once through a 2 mm hole plate. 500 g batches were adjusted to the appropriate a_w as follows: nitrite curing salt, the right amount of common salt and glucose was made up to 500 g with meat and mixed in a mixer. 5 batches with 99 g mixture were prepared and inoculated with 1 ml of the starter culture. The microorganisms were carefully worked in by hand using disposable gloves. The mixture was evacuated to remove air inclusions and put in disposable plastic containers closed with moisture impermeable lids. Measurements were taken until the pH had droped to its minimum value.

The acidification performance represents the pH drop per hour (-pH/h) and was calculated from the slope of a regression straight line approximated to the upper part of the acidification curve (Fig. 3). It includes the values from the end of the lag phase until a pH of 5.2 to 5.3 is reached. The length of the lag phase was obtained from the acidification curves (pH as a function of time) shown in Fig. 3 and 4, as the period from the start of the experiment (hour 0) to the beginning of the pH decrease. Further details are given in LANDVOGTand FISCHER [1991 a, 1991 b].

DAVEY [1989] presented a predictive mathematical model to describe the additive effects of temperature and water activity during the growth phase. He applied his model on data collected from 7 publications and demonstrated that 92.9 ... 99 % of the data variation could be explained with this model (average 96.6 %). The model is derived from a linear Arrhenius equation with additional terms for the a_w influences. In the present study the growth rate has been replaced by acidification performance ap (-pH/h) to adapt Davey's model.

$$\ln ap = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 \cdot a_w + C_4 \cdot a_w^2 \qquad (1)$$

T = absolute temperature. DAVEY [1991] extended his model to describe also the lag phase changes. According to DAVEY, ln (1/lag time) is calculated with

$$\ln (1 / \text{lag time}) = C_0 + \frac{C_1}{T} + \frac{C_2}{T^2} + C_3 \cdot a_w + C_4 \cdot a_w^2 \qquad (2)$$

The goodness of fit is discribed by % variance. Because %V takes into account both the number of data (n) and the number of terms (N) in the model it is used in addition to the multiple regression coefficient (R^2).

Results and Discussion

Because of the comprehensive experimental design with 4 starter cultures tested at 5 different temperatures and 8 different a_w values, 200 acidification curves with roughly 3000 readings have been evaluated. Fig. 3 shows the pH changes which *L. curvatus 3* creates in batches with constant a_w of 0.962 but at different temperatures. This basic plot demonstrates already a recognizable influence of temperature on acidification. If the temperature drops from 30 °C to 10 °C the time necessary to reduce the pH to 5.2 increases from 17 to 210 hours. As expected the lag phase extends also with reduced temperature.

Fig. 4 demonstrates very well the influence of different water activities on the acidification. Comparable effects like in Fig. 1 can be observe here as well: decreasing a_w increases the lag phase, the acidification performance drops and the level of minimum pH raises. The very fast acidification observed at a_w 0.993 is not only determined by the high a_w but probably also by the absence of inhibiting nitrite.

The acidification performance was calculated from the acidification kinetics as described above. Acidification performance, temperature and water activity were then worked into the Davey equation (1) to calculate the values for C_1 to C_4. The results are presented in Table 1. The variance values in Tab. 1 demonstrate how good the model fits the measured data. The average % V = 0.969 is in the same range of DAVEY's results applied to growth rates. These results are valid for a wide range of temperatures (10...30 °C) and a_w values (0.904...0.993). As the ranges chosen for temperature and a_w exceed values usually practised during dry sausage ripening, only intrapolation is neccessary to predict data which increases the reliability of the model further.

Table 1 also includes the results for the adaptation of the lag phase data of the individual starter cultures to the model. It has to be noted that in common with DAVEY [1991] the $1/T^2$ term of the model equation (2) was not significant for these data, whereas the term aw^2 was significant and had to be included in the calculation. The model fits comparably well to the lag phase results with a % variance of 0.939.

As it is an empirical model, DAVEY could not offer any physiological interpretation for the significance for the regression coefficients of Table 1. Nevertheless it should be understood that the coefficient values represent also the influence of other factors present during the test, like the substrate variation, the different lots of starter cultures, the pH, nitrite curing salt etc. As LANDVOGT and FISCHER [1991] showed, the acidification performance is independent from the glucose concentration and the degree of comminution.

but depends on the inoculation count of the starter cultures. Higher counts reduce the lag phase and increase the acidification performance.

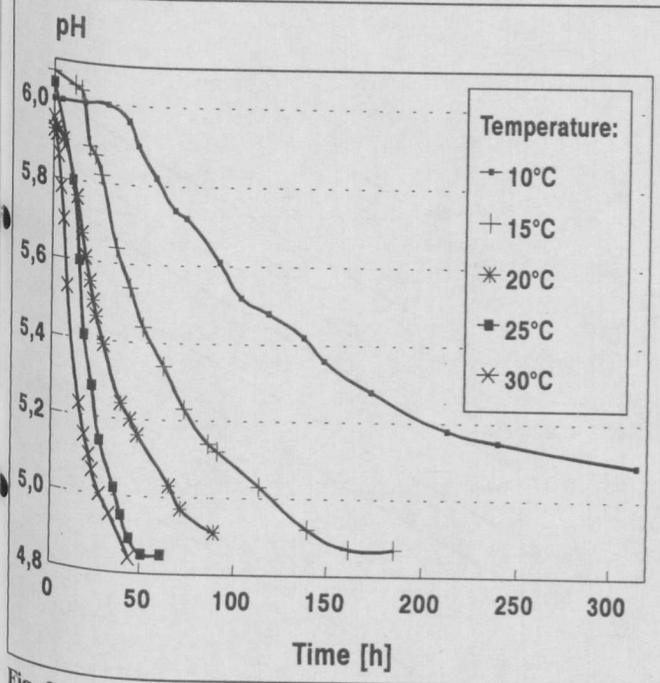

Fig. 3. acidification kinetics of *L. curvatus 3* at a_w 0.962 and various temperatures.

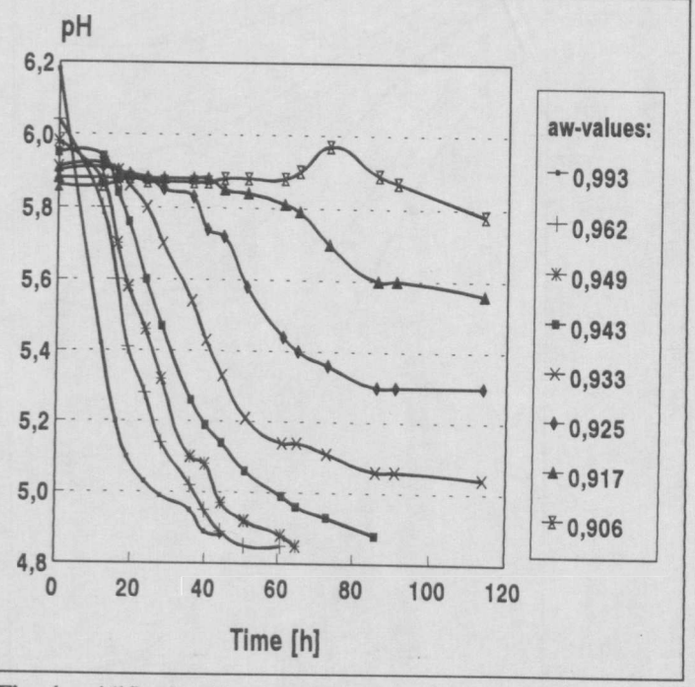

Fig. 4. acidification kinetics of *L. curvatus 3* at 25 °C and various water activities.

The kind of sugar (glucose, lactose, dextrines) will have an influence on the acidification performance but has not been tested here. In continous production processes such as dry sausage manufacturing, additional influence will stay uniform and will keep the coefficients of the model more constant. It is evident, that the water activity at 30 °C influences the acidification performance more that at 10 °C. At 30 °C the the slope of the curves at different a_w values is steeper than at 10 °C. Here the effect on acidification performance is almost equally small for all starters. As the acidification performance is already low due to the low temperature, the low a_w value scarcely affects the process.

Table 1. Fit of the model for acidification performance and lag phase(se = standard error, a.p. = acidification performance, n.s. = not significant).

Microorganism	C0	C1	C2	C3	C4	R^2	%Variance accounted for	n	F-value	se a.p.
acidification performance										
Lactobacillus curvatus 3	-968,00	1,02E+5	-1,60E+7	1676,23	-873,70	0,984	0,981	29	386,20	0,123
Lactobacillus curvatus 2	-735,41	6,11E+4	-9,82E+6	1306,91	-669,78	0,973	0,966	21	152,58	0,155
Pediococcus pentosaceus	-357,54	-1,07E+5	1,47E+7	1126,11	-579,26	0,961	0,951	20	98,49	0,150
Pediococcus acidilactici	-506,24	-2,78E+4	3,07E+6	1147,08	-586,25	0,981	0,976	19	198,60	0,119
lag-Phase										
										se lag-Phase
Lactobacillus curvatus 3	-312,84	-9,11E+3	n.s.	674,98	-332,28	0,986	0,985	36	764,48	0,165
Lactobacillus curvatus 2	-614,36	-8,38E+3	n.s.	1304,89	-664,13	0,917	0,906	28	84,31	0,295
Pediococcus pentosaceus	-475,00	-1,12E+4	n.s.	1018,10	-506,56	0,967	0,963	28	241,68	0,263
Pediococcus acidilactici	-325,22	-1,03E+4	n.s.	702,48	-343,91	0,915	0,903	28	82,17	0,404

This means that the acidification performance at low a_w values is almost independant from the chosen temperature, because the water activity has taken over the dominat role as an inhibiting factor.

As demonstrated, the model describes very well the ripening process. The logical next step based on this learning would be a targeted control of the fermentation. It is planned to measure continuously the pH during the first days of fermentation. Together with the continuously measured temperature and a_w of the ripening sausage, it is possible to build up a closed loop control. By adjusting the air temperature the control loop influences the acidification performance of the starters and therefore the acidification can be controlled [LANDVOGT, 1992]. The above equation to calculate the actual acidification performance is essential to compare the actual state of the fermentation with the desired one. Does the actual result for acidification differs from the previous set point, a temperature readjustment based on the equation can be initiated which compensates the difference.

The benefits gained by transferring the predictive microbiology methods to fermentation processes with starter cultures seem even more promising than growth rate descriptions for food spoiling species.

Microbiological cultures which are used commercially as starters exist only in a limited number of strains. The strains are identified and are offered in a pure form and under standardized conditions. This allows a clear analysis of their acidification kinetics and eliminates, in case of dominat cultures, any influence of spontaneous flora on the acidification.

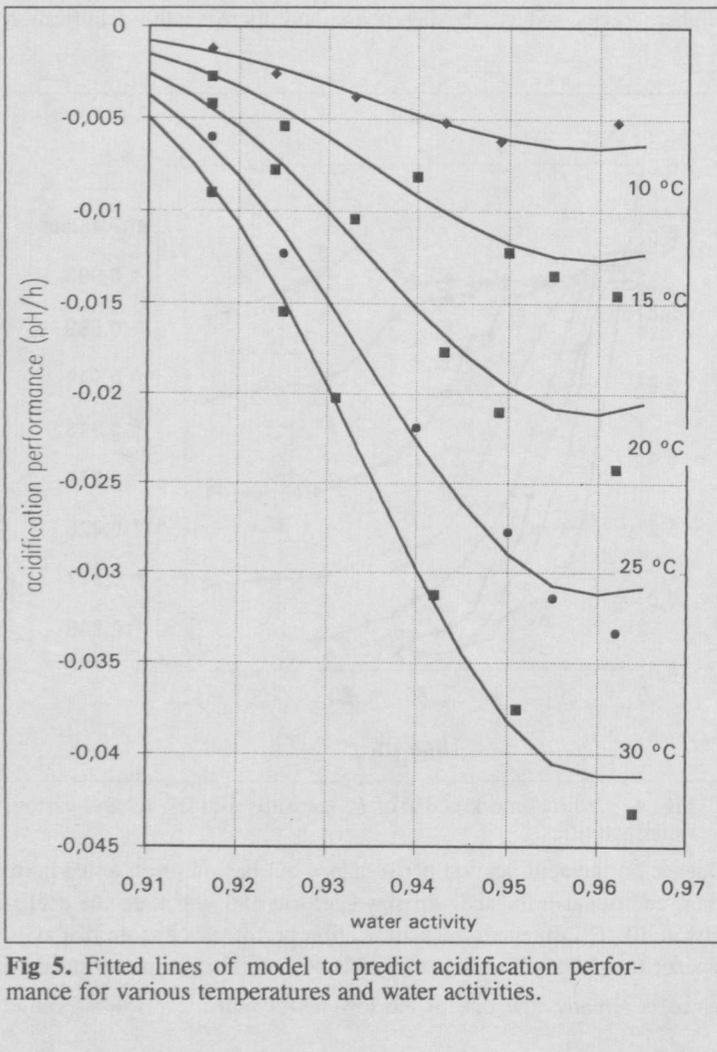

Fig 5. Fitted lines of model to predict acidification performance for various temperatures and water activities.

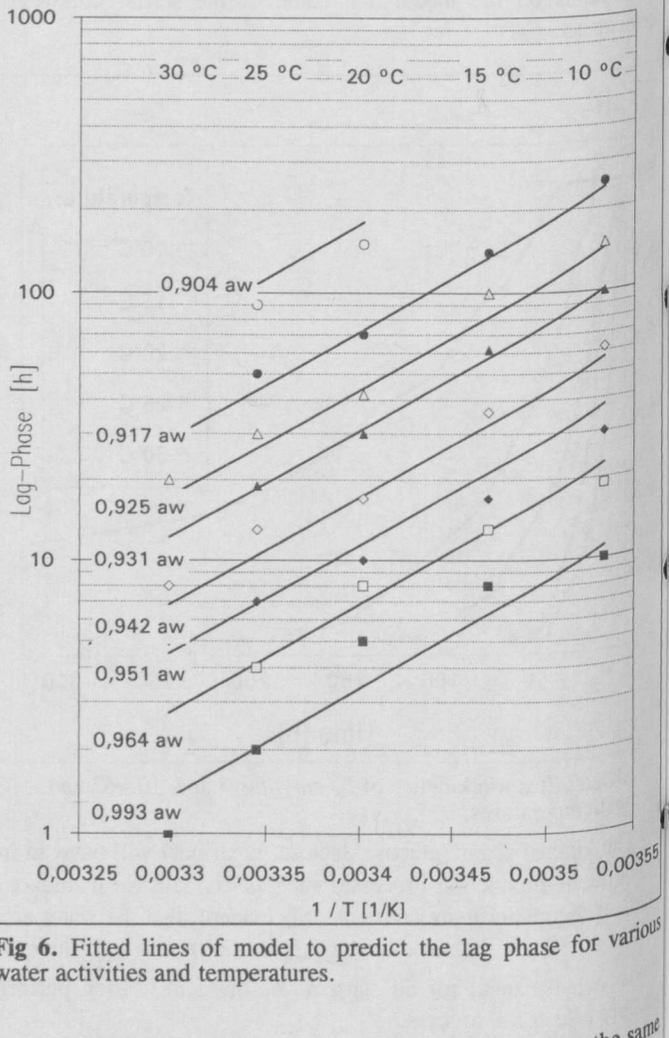

Fig 6. Fitted lines of model to predict the lag phase for various water activities and temperatures.

In most cases the substrate for starter cultures is very well known and standardized by the recipe. This allows to reproduce the same test conditions specially as the history of the substrate is known.

The kinetics of starters depend only on a few parameters of which most can be standardized by the recipe. For further investigations during industrial processing the influence of added spices, different sugars and combined starter preparations can be incorporated in the substrate to improve the reliability of the results.

Conclusions

The principles and methods of predictive microbiology can be extended to starter cultures in addition to food spoiling microorganisms. Instead of growth rates the fermentation product can be continuously measured which is a prerequisit to make targeted control possible for food fermentation.

As foods (instead of artificial growth media) are used as substrate, it is easier to transfer the results and validate them in commercially utilized processes. The combined influence of parameters can be analyzed.

An empirical mathematical model has been successfully applied to model the acidification of dry sausage; it can be included in a control loop to control the acidification performance by temperature. Additional applications for fermentation process as joghurt-, sour dough-, Sauerkraut- and winemaking may be possible.

References

DAVEY, K. R. (1989): A predictive model for combined temperature and water activity on microbial growth during the growth phase. Journal of Applied Bacteriolgy, (67), 483-488

DAVEY, K. R. (1991): Applicability of the Davey (linear Arrhenius) predictive model to the lag phase of micobial growth. Journal of Applied Bacteriology (70), 253-257

LANDVOGT. A. (1992): Controlled ripening and drying concept for dry sausage manufacturing. Proceedings of the IChemE Conference: Food Engineering in a computer climate. IChemE Symposium series No. 126, Hemisphere Publishing Corporation ISBN1-56032-255-1, pp 291-296.

LANDVOGT, A. and FISCHER, A. (1991 a): Dry sausage ripening- Targeted control of the acidification achieved by starter cultures. Fleischwirtschaft 71 (8), 902-905

LANDVOGT, A. and FISCHER, A. (1991 b): Dry sausage ripening- Targeted control of the acidification achieved by starter cultures, Part 2. Fleischwirtschaft 71 (9), 1055-1056

LEISTNER, L. (1990): Produktsicherheit durch Anwendung des HACCP-Konzeptes und der Voraussagenden Mikrobiologie. In: Sichere Produkte bei Fleisch und Fleischerzeugnissen. Institut für Mikrobiologie, Toxilkologie und Histologie der Bundesanstalt für Fleischforschung, Kulmbach (Herausgeber). pp 201-222