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Short Communication

A quantitative method for predicting shelf life of soft drinks using a model system

M.B. Cole and M.H.J. Keenan

AFRC Institute of Food Research, Norwich, NR4 7UA, U.K.

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SUMMARY

A quantitative method for the prediction of growth of the food spoilage yeast *Zygosaccharomyces bailii* in a model fruit-drink system is described. A factorially designed experiment was employed to produce polynomial equations relating pH and sugar concentration (°brix) to the lag period and doubling time of this yeast. Low pH values (< 3.0) and high °brix values (> 40) show a strong synergistic action on the extension of lag period, which could be used, along with the model presented, in the formulation of product preservation systems.

INTRODUCTION

Involvement of the yeast *Zygosaccharomyces bailii* in the spoilage of food products which combine a low pH and a high sugar °brix, such as fruit beverages, is well documented [8]. Moreover, incidence of spoilage caused by this organism appears to be on the increase [3], and this may be correlated with an attempt within the food industry to reduce or eliminate the use of weak-acid preservatives from such products [5].

It is also well known that reducing available water narrows the pH limits for bacterial growth [6,9] and has similar effects on yeasts and moulds [4,10]. Whether there is synergistic action between these two factors is unknown. However, in order to assess the hygiene regimes necessary to accommodate systems relying upon pH and °brix alone, quantitative data on the effect of these two factors on the growth of *Z. bailii* are necessary. The present work uses the technique of factorial experiments [1,2] to quantify individual effects and interactions between pH and °brix on both doubling time and lag period of *Z. bailii* in a model fruit beverage system.

Correspondence: Dr. M.B. Cole, AFRC Institute of Food Research, Norwich, NR4 7UA, U.K.

MATERIALS AND METHODS

Organism

Z. bailii (NCYC 563) was obtained from the National Collection of Yeast Cultures, AFRC In-

stitute of Food Research, Norwich Laboratory, Colney Lane, Norwich, U.K.

Growth

Starter cultures were shaken (100 rpm) for 2 h at 23°C, pH 4.5, after which time 6×10^6 organisms were inoculated into experimental flasks. Growth was measured turbidometrically using a Unicam SP600 Spectrophotometer (E 600_{nm}–1 cm) and readings were compared with calibration curves of optical density plotted against cell mass or cell number. Samples with optical densities greater than 0.5 were diluted with distilled water.

Media

Media contained Yeast Nitrogen Base (Difco, Detroit, U.S.A.) (6.7 $g \cdot l^{-1}$) and fructose (50 $g \cdot l^{-1}$) and were buffered with a combination of citric acid (0.2 M) and di-potassium hydrogen orthophosphate (0.6 M) to achieve pH values between 2.5 and 4.0. Solutions of yeast nitrogen base in citrate/phosphate buffer and fructose were autoclaved separately and then combined.

EXPERIMENTAL DESIGN AND ANALYSIS

In a symmetrically designed orthogonal factorial experiment, concentrations of the factors (fructose and hydrogen ions) were set at all combinations of low-, medium- and high-factor levels. Hydrogen-ion concentrations were set at 10 μM (pH 4.0), 1.62 mM (pH 2.79) and 3.16 mM (pH 2.5). Fructose concentrations were set at 200 $g \cdot l^{-1}$ (20 °brix), 375 $g \cdot l^{-1}$ (37.5 °brix) and 550 $g \cdot l^{-1}$ (55 °brix).

The effects of factors on doubling time (four independent replicates) were analysed for a least-squares fit of a quadratic model, and those for lag period to an exponential function of a quadratic model using Genstat V (Nelder, 1973) (copyright 1984, Lawes Agricultural Trust, Rothamsted Experimental Station), on a Vax mini-computer (AFRC, Agrenet node FRIN).

Measurement of °brix and pH throughout growth

°Brix and pH were measured using a refractometer (Bellingham and Stanley Ltd, London) and a Phillips PW9410 digital pH meter, respectively.

RESULTS

Measurement of °brix and pH throughout growth

°Brix varied by no more than 5% as measured by refractometry throughout the duration of the experiment, whilst pH remained constant (Table 1).

Table 1

Doubling times and lag periods for *Zygosaccharomyces bailii* NCYC 563 at different °brix and pH values

°Brix	Hydrogen-ion concentration (mM)	Doubling time (h)		Lag period (h)	
		Mean	S.D. ($n=4$)	Mean	S.D. ($n=4$)
20.0	0.10 (pH 4.0)	2.96	0.03	12.09	0.44
37.5	0.10 (pH 4.0)	5.04	0.11	27.16	0.76
55.0	0.10 (pH 4.0)	7.86	0.42	136.31	4.73
20.0	1.62 (pH 2.79)	5.02	0.03	8.66	0.70
37.5	1.62 (pH 2.79)	9.72	0.58	57.30	4.24
55.0	1.62 (pH 2.79)	26.04	1.18	506.24	7.21
20.0	3.16 (pH 2.5)	7.16	0.63	16.78	3.88
37.5	3.16 (pH 2.5)	21.09	1.14	60.93	6.72
55.0	3.16 (pH 2.5)	25.91	2.92	745.90	30.29

Statistical analysis (two-way factorials)

Two-way factorial analyses produced polynomial equations linking °brix [F] and hydrogen-ion concentration [H^+] (mM) to doubling time (Dt) (h) and to an exponential function (x) of lag period (Lag) (h).

$$Dt = -2.47 + 8.30\times10^{-2}[F] + 2.07[H^+] - 1.80\times10^{-3}[F]^2 - 8.40\times10^{-1}[H^+]^2 + 1.29\times10^{-1}[F][H^+] \quad (1)$$

$$Lag = e^x$$

where

$$x = 2.14 - 1.67\times10^{-2}[F] - 2.90\times10^{-2}[H^+] + 1.25\times10^{-3}[F]^2 - 4.70\times10^{-2}[H^+]^2 + 1.30\times10^{-2}[F][H^+] \quad (2)$$

DISCUSSION

In our model system, the effects that pH and °brix have on the doubling time of *Z. bailli* can be expressed as a polynomial equation (equation 1) including terms for individual-linear and quadratic effects as well as for an interaction between the two. Here, the individual contributions are very strong and the synergism quite weak (Fig. 1a). The model for lag period, on the other hand (equation 2), is related to an exponential function in which the interaction between pH and °brix becomes increasingly important, with only °brix having any marked effect when acting alone (Fig. 1b).

The consequence of this synergistic action is that while little advantage in terms of an extended lag period is gained until about 40°brix and pH 3.0, any subsequent increase in °brix and/or decrease in pH has a dramatic effect on lag period, such that at the most inhibitory conditions of 55 °brix and pH 2.5 it would take an initial contamination level of 1–2 cells·ml^{-1} over a month to reach detectable spoilage levels (10^4 cells·ml^{-1}).

In taking this approach to shelf life prediction by constructing these or similar experiments, with the actual product, some caution must be exercised. We have found (Cole and Keenan, unpublished observations) that although doubling time of *Z. bailii* is determined only by growth conditions, the lag period will vary depending upon the prehistory of the inoculum. In the plant this factor will be beyond control. Nevertheless, providing that care is taken to determine where a given combination of °brix content and pH level are on the interaction surfaces, it is possible to predict accurately the shelf life of a product.

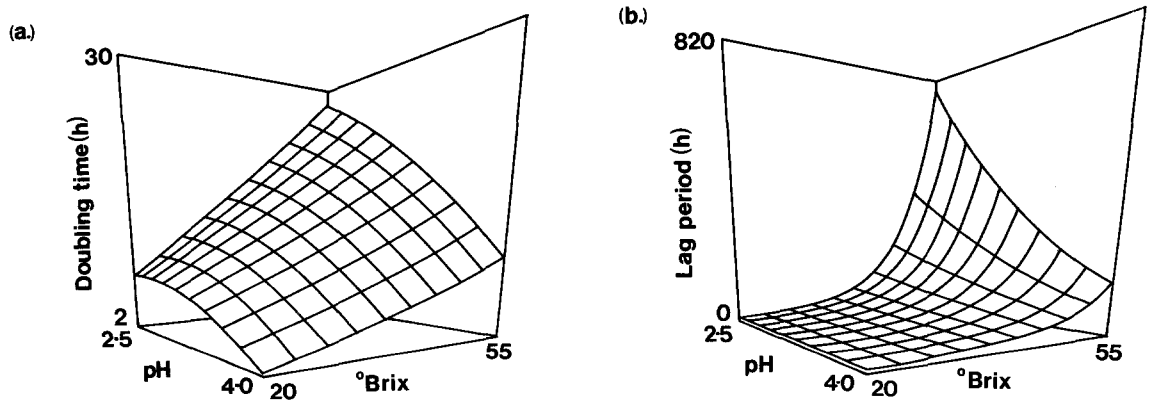

Fig. 1. The effect of pH (x axis) and °brix in terms of fructose concentration (z axis) on population doubling time (y axis, (a)) and lag period (y axis, (b)), of *Z. bailii* NCYC 563. The response surfaces were generated from full factorial analyses of the orthogonal experiments described in Materials and Methods.

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