Journal of Industrial Microbiology, 12 (1993) 301–308

Published by The Macmillan Press Ltd

Use of indirect conductimetry for predicting growth of food spoilage yeasts under various environmental conditions

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(Received 25 February 1993)

Key words: Indirect conductimetry; Food spoilage yeasts; Predictive modeling

SUMMARY

Four variables (temperature, a_w, pH and potassium sorbate concentration) at three levels were studied to determine their effects on the growth of six yeasts (*Candida glabrata, Candida parapsilosis, Debaryomyces hansenii, Pichia membranaefaciens, Saccharomyces cerevisiae* and *Zygosaccharomyces bailii*) isolated from spoiled food products. The detection time (DT) and the maximum change in conductance (MRC) were measured by indirect conductimetry using a Malthus instrument. Temperature, a_w and potassium sorbate concentration were the most important variables individually and in combination that affected yeast growth. Shelf life of fruit juice at $a_w \leq 0.96$, pH ≤ 3.8 and containing $\leq 0.03\%$ potassium sorbate, when stored at ≤ 10 °C, would be predicted to be greatly extended. *Z. bailii* was the most resistant of the yeasts in terms of ability to tolerate stress conditions and is proposed as a test species to develop a predictive model for spoilage.

INTRODUCTION

The shelf life of many foods depends on a combination of factors such as pH, a_w, preservatives, temperature and time. For a wide range of foods and beverages with high sugar content and low pH, with or without preservatives, yeasts are often the most common spoilage microorganisms [5,12,26]. Much effort has been directed toward determining the effect of single factors such as pH [25], preservatives [23,34] a_w [20,33], CO_2 [18,30] and temperature [3,27] on growth of yeasts. Although a synergism between multiple factors would enable milder preservation treatments and result in improved product quality, only a few studies have been done to determine the combined effect of two or more factors on survival and growth of yeasts. The combined effects of type of acid and pH [7], preservatives and temperature [4], a_w and temperature [11,19], a_w and preservatives [22] have been studied. Beuchat [4] studied the inhibitory effect of preservatives, a_w and temperature, while Cole and Keenan [8] investigated the effect of pH, a_w, preservatives and temperature.

A lack of definitive information on the combined effect of factors which may be potentially inhibitory to yeast growth is partially due to difficulties associated with traditional plating techniques. To overcome these difficulties, [8] applied turbidimetry, [9] used a microtiter-plate system, [22] assessed gas production visually and [16] monitored CO_2 development by gas chromatography.

In recent years, there has been an increased interest in improving methods for the detection of yeasts in foods and beverages [5,12,21]. Rapid and automated techniques have become generally available that make the determination of microbial populations in a large number of samples within a short time more attainable [1,31]. One of these non-traditional techniques is electrometry, i.e. measurements of conductance, capacitance or impedance, that can be used to detect a wide range of bacteria, yeasts and molds in foods [14,35]. Contrary to bacteria which tend to increase the conductance of growth media, yeasts tend to reduce conductance [10,29]. Electrometric techniques have been used to detect yeasts in alcoholic beverages [2,17], fruit products [15,36] and various other foods [29,35].

Although gas production by some yeasts interferes with electrical measurements [10,17], recently a method described as indirect conductimetry has been developed [24] that is based on CO_2 evolution as an indication of yeast activity. The CO_2 is absorbed in an alkaline solution and the rate of production is measured conductimetrically. This indirect method offers an advantage over conventional conductimetry in that the electrical signal is greatly magnified and analysis can be made directly in the food sample without addition of any medium [6].

The study reported here was done to evaluate the effect of temperature, a_w, pH and potassium sorbate concentration on growth characteristics of selected food spoilage yeasts.

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Mention of brand or firm names does not constitute an endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

TABLE 1

Yeast species studied

Species	Strain no.	Origin
Candida glabrata	Y00581	Spoiled fruit juice
Candida parapsilosis	Y00541	Spoiled fruit juice
Debaryomyces hansenii	Y00313	Spoiled mayonnaise
Pichia membranaefaciens	Y00635	Spoiled fruit juice
Saccharomyces cerevisiae	CBS1395	Fermenting must
Zygosaccharomyces bailii	Y00534	Turbid wine

Indirect conductimetry was used to monitor yeast activity and response surface methodology was applied for evaluation.

MATERIALS AND METHODS

Procedure for preparing media and cells

The yeast strains selected for investigation were originally isolated from spoiled foods (Table 1). Cultures were obtained from the National Collection of Agricultural and Industrial Microorganisms (NCAIM), Budapest, Hungary. Yeasts were grown on potato dextrose agar (pH 5.5) at 30 °C for 24–48 h. Colonies were picked and suspended in sterile 0.1% peptone; cell populations were estimated using a hemocytometer and then diluted to about 2×10^6 ml^{-1}. These suspensions served as inocula for indirect conductimetry studies using apple juice media.

Commercial apple juice was used as the basal medium to which sucrose was added to adjust a_w to desired values (0.93, 0.96 and 0.99). After dissolution of sucrose, the pH was adjusted to 3.8, 4.2 and 4.6 by addition of 0.1 N HCl or 0.1 N NaOH. Potassium sorbate (20% w/v) was added to yield concentrations of 0,0.03 and 0.06%. The a_w was determined using a Rotronic hygroscop DT instrument (Zurich, Switzerland) and the pH was determined using a Corning digital 112 pH meter (Medfield, MA, USA).

Apple juice media were dispensed (4 ml) into Malthus (Malthus System, Inc., Crawley, Sussex, UK) CO_2 cells containing 0.5 ml of 0.1 M KOH in the insert chamber, and 0.05 ml of yeast suspension was added to give a population of about 10^5 ml^{-1} as determined by spread plating 0.1 ml of suspensions serially diluted in 0.1% peptone on tryptone yeast extract glucose agar (pH 5.5). Colonies were counted after incubating plates at 25 °C for 5 days. The inoculated CO_2 cells were immediately placed into the Malthus incubator set at 10, 20 or 30 °C, and scanning and recording was done automatically by a Malthus 2000 instrument.

Experimental design and data analysis

The experimental variables and levels are presented in Table 2. A full factorial, four variable, three-level experimental design with three replicates was used to study *Zygosaccharomyces bailii*, while a full factorial, three-variable (a_w, pH, potassium sorbate), three-level experiment with two replicates was done for each of the three temperatures for *Candida glabrata, Candida parapsilosis, Debaryomyces hansenii, Pichia membranaefaciens* and *Saccharomyces cerevisiae*.

TABLE 2

Experimental variables

Variable	Factor	Level 1	2	3
Temperature (°C)	x_1	10	20	30
Water activity	x_2	0.93	0.96	0.99
pH	x_3	3.8	4.2	4.6
Potassium sorbate (%)	x_4	0.00	0.03	0.06

Two dependent variables, detection time (DT) and the maximum rate of change (MRC) in conductance, were monitored to characterize yeast activity and growth. DT was recorded instrumentally using Malthus software, and MRC was calculated manually from the slope of the conductance curve as illustrated in Fig. 1 for *Z. bailii*.

The RSREG procedure of Statistical Analysis System [28] was used to fit the second order polynomial equation to experimental data. Polynomial equations which were significant at $P \leq 0.15$ level were used to develop response surface plots. Contour plots and surface plots were generated by a Surfer program (Golden Software, Inc., Golden, CO, USA) for each parameter as a function of two independent variables while the other variables were held constant.

RESULTS AND DISCUSSION

Evolution of CO_2 can be used to monitor microbial growth [13]. Measurement of CO_2 by gas chromatography

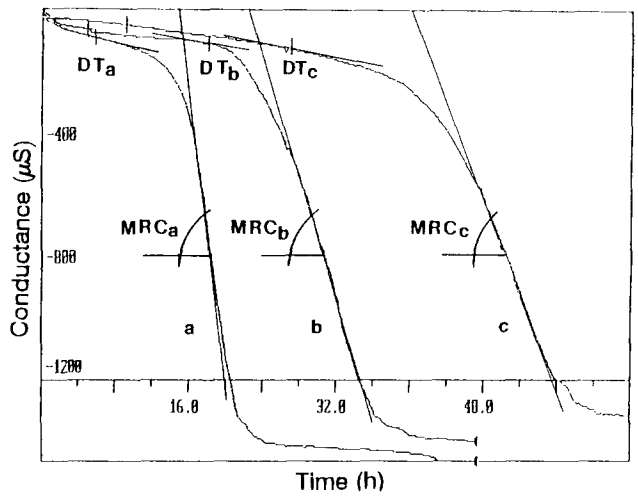

Fig. 1. Conductance curves and determination of DT and MRC values. *Z. bailii* was incubated at 30 °C in apple juice (a_w 0.96, 0.06% potassium sorbate) at (a) pH 4.6, (b) 4.2 and (c) 3.8. DT is expressed as hours required to reach a rate of conductance change of 5 μS h^{-1}. MRC is expressed in μS h^{-1} as the tangent of the slope of the curve.

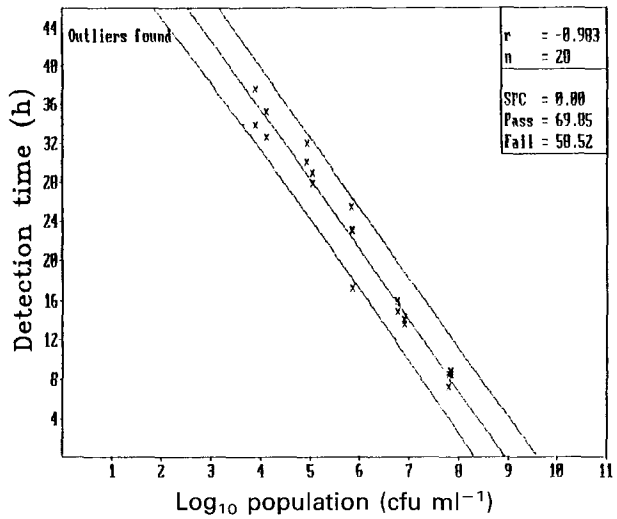

Fig. 2. Calibration curve for DT versus $\log_{10}$ population. *Z. bailii* was inoculated at various populations (as confirmed by plating) into apple juice (a_w 0.99, pH 4.6, 0.0% potassium sorbate) and incubated at 30 °C in Malthus CO_2 cells.

has been applied to determine the effect of combined environmental factors on activity and growth of yeasts [16]. Indirect conductimetry is another method for detecting CO_2 evolution during growth, and has been used successfully for detection of some foodborne bacteria [6]. In agreement with Owens et al. [24], our results show that indirect conductimetry can be used to detect CO_2 production by viable yeast cells and to subsequently monitor growth. Growth resulted in decreased conductance (Fig. 1), the extent of change strongly correlating in terms of DT and MRC with the size of yeast population (Fig. 2). (For simplicity, not all data from all test yeasts are presented.) The maximum change in conductance was well over 1300 μS, which is 3–4 times more than that obtained by conventional conductimetry in an optimal medium [10].

Well fitting polynomial prediction model equations for DT (Table 3) and MRC (Table 4), describing response surfaces, were generated from conductance data. The growth of yeasts expressed as DT and MRC is influenced by test factors in linear and quadratic terms as well as by complex interactions between factors. The most significant factors individually affecting DT and MRC were a_w and potassium sorbate (KS) concentration, as well as interactions of temperature (TE) in all combinations (KS × TE, pH × TE, a_w × TE) and interaction between KS and a_w. The pH exerted a significant effect only in interactive terms with other factors. Similar polynomial equations to determine the effects of complex interactions between environmental factors on yeast growth have been produced [9,16].

Figure 3 illustrates the interactive effects of a_w and potassium sorbate on *S. cerevisiae*. A combination of increased potassium sorbate and sucrose concentrations resulted in increased DT and decreased MRC more than individual factors did. The combined effects on MRC were less pronounced as the incubation temperature was increased (Fig. 4). Higher MRC, analogous to the specific growth rate constant, indicates a more rapid growth rate, whereas higher DT values indicate slower growth rates, although an analogy between DT and the lag phase of growth is plausible.

Our data show that the most significant factors affecting yeast growth in terms of conductance change were a_w potassium sorbate concentration and temperature. Cole et al. [9] described a synergism between a_w (°Brix) and sorbic acid on the growth of *Z. bailii*. In our study, synergism between inhibitory factors was observed for some yeasts, e.g. *S. cerevisiae, C. glabrata, P. membranaefaciens* and *Z. bailii*. For *C. parapsilosis* and *D. hansenii*, the potassium sorbate concentration alone was a limiting factor to the

TABLE 3

Best selected prediction models for detection time (DT)

Yeast	Equation[a]	r^2	F[b]
Candida glabrata	DT = − 9399 + 21375AW + 1.4PH − 12118KS − 1.6TE − 111757AW² − 17155KS² + 0.1TE² − 7.6AWTE + 1414AWKS − 1.2PHTE + 34KSTE	0.87	0.87
Candida parapsilosis	DT = 28 + 221AW + 79PH + 4184KS + 0.8TE − 44395KS² − 97AWPH − 7.3AWTE	0.89	0.99
Debaromyces hansenii	DT = 14981 − 30352AW − 193PH + 13221KS − 12TE + 15074AW² − 32PH² − 37305KS² + 0.1TE² − 10723AWKS + 472AWPH + 28KSTE	0.87	1.52
Pichia membranaefaciens	DT = − 8930 + 19398AW − 2.6PH − 6595KS + 32TE − 10161AW² − 14562KS² + 0.1TE² − 42AWTE + 7700AWKS − 1.2PHTE + 39KSTE	0.89	1.75
Saccharomyces cerevisiae	DT = −9318 + 20720AW + 7.1PH − 906KS + 8.4TE −11166AW² + 0.2TE² − 16AWTE + 444PHKS − 2.3PHTE	0.84	5.84[c]
Zygosaccharomyces bailii	DT = 2303 − 1934AW − 19PM − 5407KS − 95TE + 0.7TE² + 7864AWKS + 65AWTE − 74KSTE	0.91	0.97

[a]AW: a_w; KS: potassium sorbate concentration (%, w/v); PH: pH; TE: temperature (°C).

[b]Significantly different values ($P<0.05$) compared to the respective full models.

[c]No reduced equation was significantly different ($P<0.05$); the full model was used: DT = − 8853 + 19500AW + 174PH − 15072KS + 7.0TE − 11166AW² − 41PH² − 12063KS² + 0.3TE² + 15122AWKS + 182AWPH − 16AWTE + 444PHKS − 2.3PHTE + 19KSTE.

TABLE 4

Best selected prediction models for maximum rate of change (MRC) in conductance

Yeast	Equation[a]	r^2	F[b]
Candida glabrata	MRC = 29958 − 62084AM − 43PH + 35160KS − 158TE + 32223AW² + 7977KS² − 35416AWKS + 157AWTE + 4.5PHTE − 177KSTE	0.80	2.14
Candida parapsilosis	MRC = 2788 − 6330AW + 6.0PH + 14167KS − 22TE + 3539AM² + 39959KS² + 0.1TE² − 14923AWKS + 21AWTE + 1.1PHTE − 389PHKS − 97STE	0.91	0.97
Debaromyces hansenii	MRC = − 16354 + 34506AW − 10PH − 6807KS − 1.8TE − 18158AW² + 15700KS² + 6481AWKS + 1.1PHTE − 47KSTE	0.59[c]	1.79
Pichia membranaefaciens	MRC = 33139 − 62045AW − 1324PH + 21261KS − 180TE + 28736AW² + 1338AWPH − 20988AWKS + 177AWTE + 4.5PHTE − 107KSTE	0.83	1.75
Saccharomyces cerevisiae	MRC = 71175 − 150465AW + 41PH + 115362KS − 241TE + 79146AW² + 57479KS² − 121358AWKS + 266AWTE − 263KSTE	0.75	0.81
Zygosaccharomyces bailii	MRC = 22323 − 4715AW − 55PH − 2381KS − 18TE + 25122AW² + 0.3TE² + 762PHKS + 4.4PHTE − 76KSTE	0.84	1.85

[a]AW: a_w; KS: potassium sorbate concentration (%, w/v); PH: pH; TE: temperature (°C).
[b]Significantly different values ($P<0.05$) compared to the respective full models.
[c]No equation was obtained above 0.70 r^2 coefficient of determination.

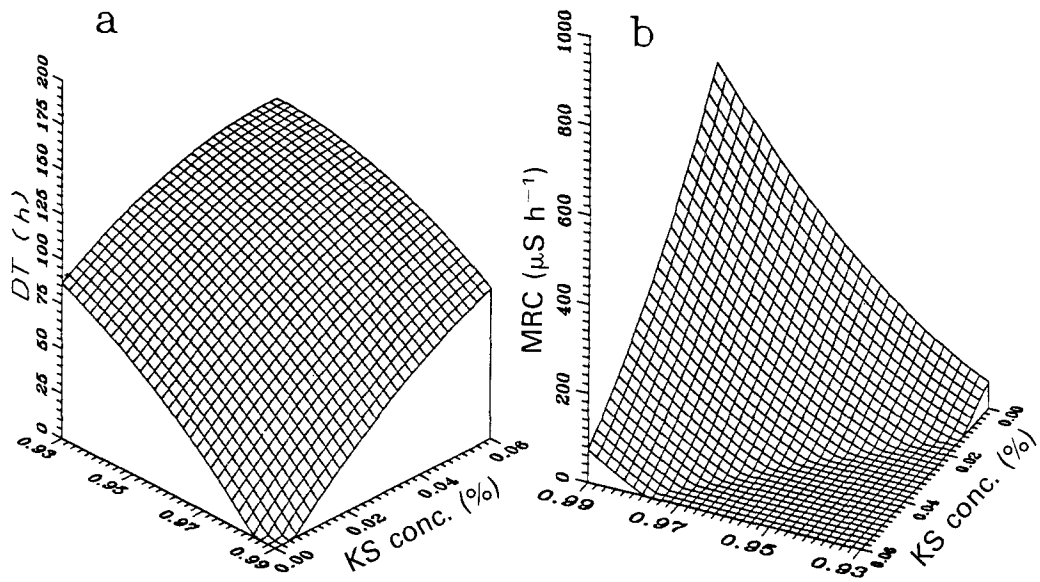

Fig. 3. Surface plots of DT values (a) and MRC values (b) for *S. cerevisiae* as a function of a_w and potassium sorbate (KS) concentration at pH 4.2 and 30 °C.

extent that it greatly reduced the impact of other stress factors on growth. The DT for *C. parapsilosis* reached a maximum value in media containing ≤0.024% potassium sorbate at 10 °C (Fig. 5a). At 20° C (Fig. 5b) and 30 °C (Fig. 5c) the interaction between a_w and potassium sorbate was more pronounced.

Surface plots (Fig. 6a) clearly demonstrate that DT values for *C. parapsilosis* depend largely on potassium sorbate concentration. This is in conformity with the large linear and quadratic coefficients for potassium sorbate in the model equation (Table 4). Model equations reveal that the two most important factors affecting growth and metabolism of *Z. bailii* are a_w and potassium sorbate concentration in linear and interaction terms; Fig. 6b illustrates a synergistic effect between these factors. It is generally recognized [32] that *Z. bailii* is among the most resistant of the yeasts to stress factors. In our experiments, too, compared to the other yeasts studied, *Z. bailii* was far more resistant to stress conditions. Changes in conductance occurred in the presence of 0.06% potassium sorbate (a_w 0.93, pH 3.8) at 20 and 30 °C (Fig. 7) Inhibition was observed at 10 °C at a_w ≤0.95 and ≤0.98 at potassium sorbate concentrations of 0 and 0.06%, respectively.

Although pH influences the dissociation of weak acids, it only slightly affects conductance changes (Fig. 8a). The interaction of pH with potassium sorbate was more apparent

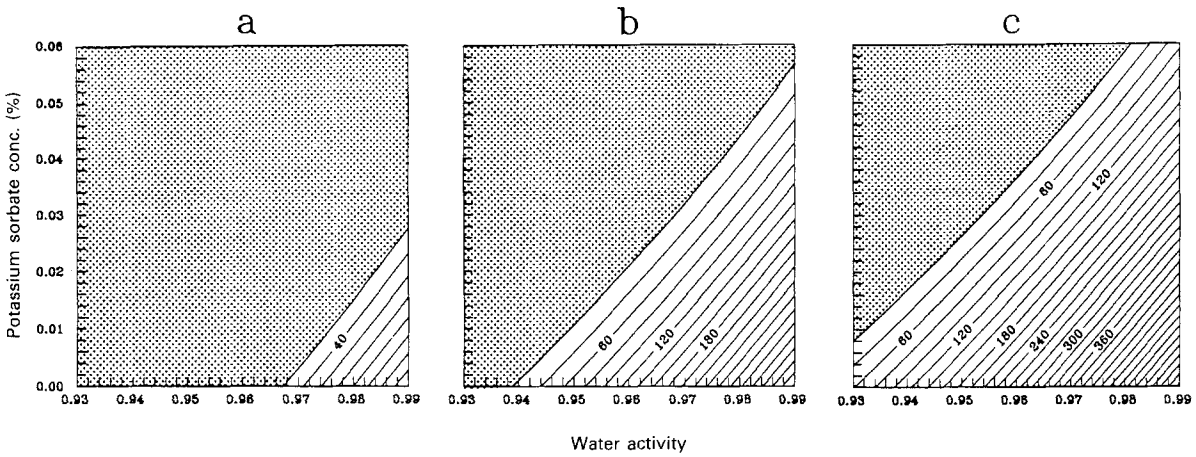

Fig. 4. Contour plots of MRC of *S. cerevisiae* as a function of a_w, potassium sorbate concentration and temperature at pH 4.2 at (a) 10 °C, (b) 20 °C, (c) 30 °C.

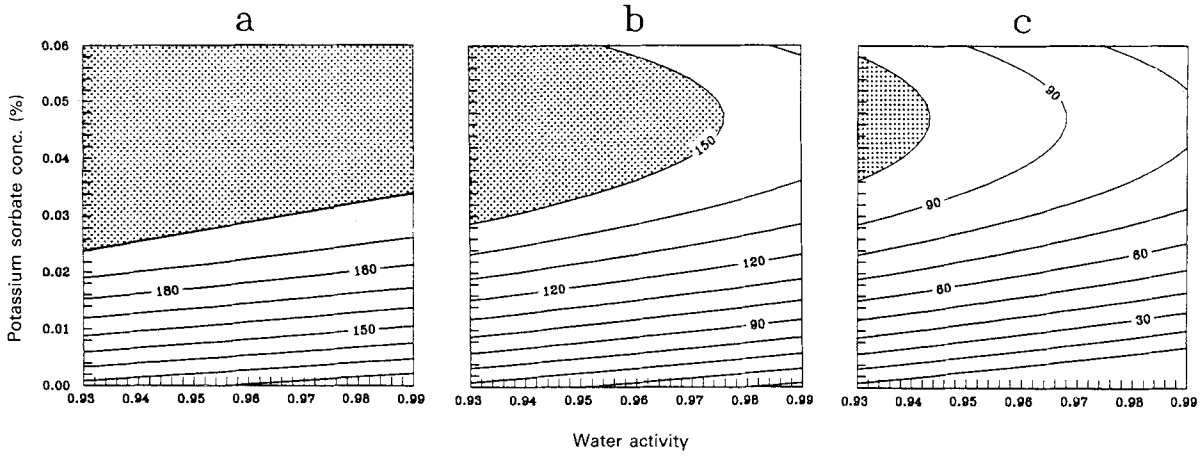

Fig. 5. Contour plots of DT of *C. parapsilosis* as a function of a_w and potassium sorbate concentration at pH 4.2 at (a) 10 °C, (b) 20 °C, (c) 30 °C.

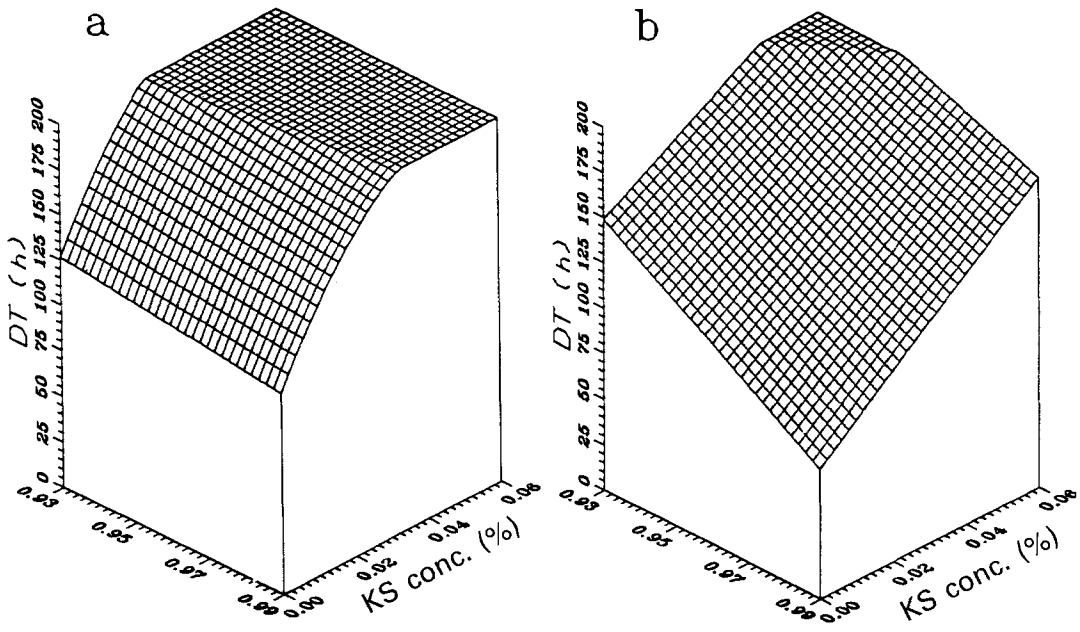

Fig. 6. Surface plots of DT values for *C. parapsilosis* (a) and *Z. bailii* (b) as a function of a_w and potassium sorbate (KS) concentration at pH 4.2 and 10 °C.

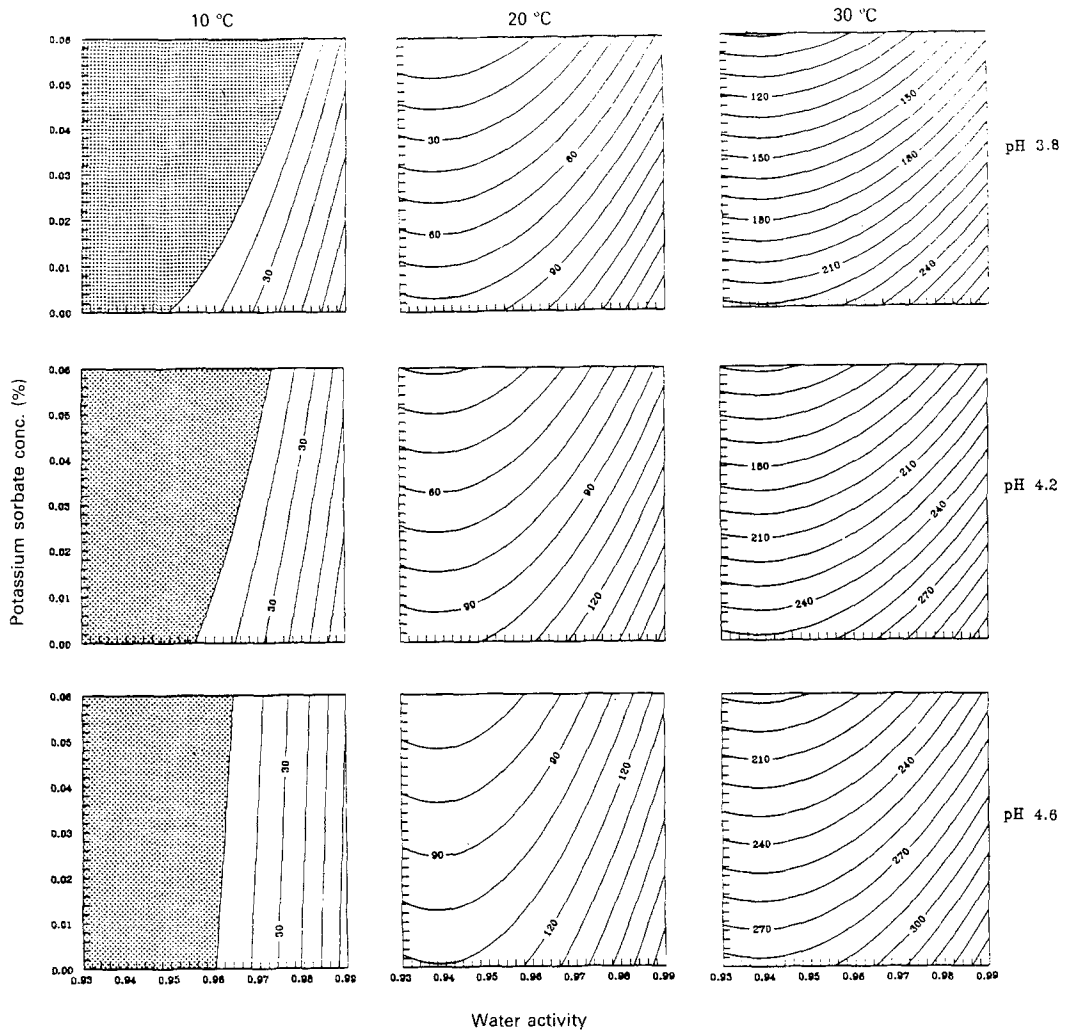

Fig. 7. Contour plots of MRC of *Z.bailii* as a function of a_w and potassium sorbate concentration at various temperatures and pH values.

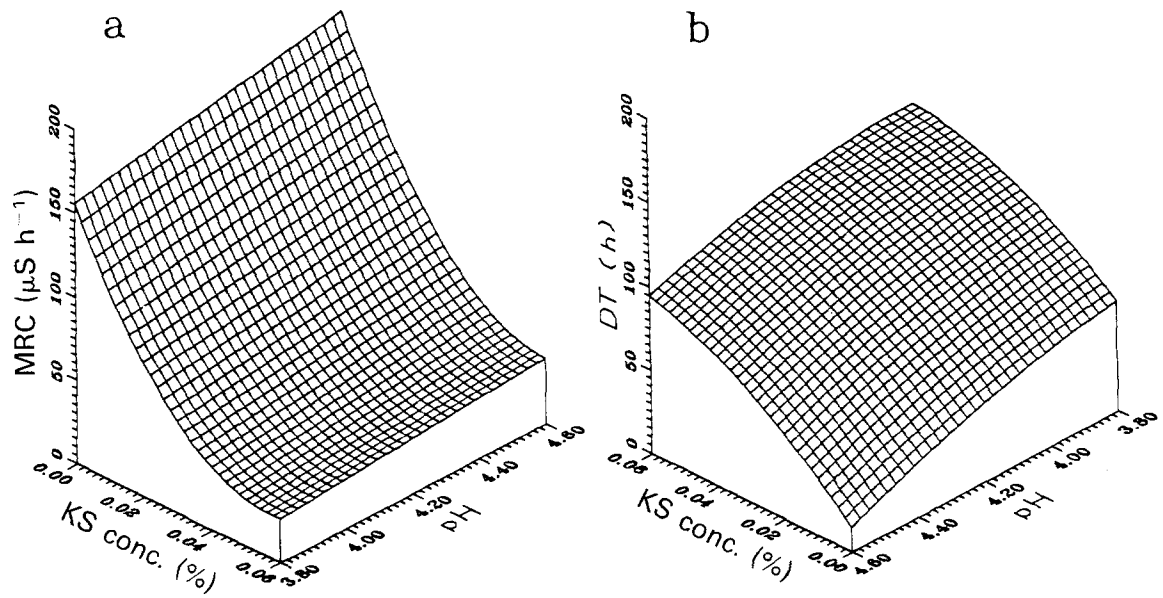

Fig. 8. Surface plots of MRC values for *C. parapsilosis* (a) and DT values for *S. cerevisiae* (b) as a function of potassium sorbate (KS) concentration and pH at a_w 0.96 and 30 °C.

at lower a_w values where it amplified inhibition by potassium sorbate (Fig. 8b). In the pH range studied, the potassium sorbate was 62–90% undissociated. Cole et al. [9], applying low pH values (2.50, 2.79), observed that a synergism between a_w and sorbic acid on the growth of *Z. bailii* was pH dependent. Guerzoni et al. [16] found, in agreement with our observations, that pH alone (in the range of 3.02–4.09) was a weaker stress factor than a_w or the preservative, benzoic acid.

As an index of growth inhibition, except for *Z. bailii*, yeasts generally did not cause changes in conductance under conditions of a_w 0.96, pH 4.2 and 0.03% potassium sorbate at 20 °C. Stress conditions were magnified as the incubation temperature was decreased to 10 °C.

These observations are in agreement with those of Maimer and Busse [22] who reported that yeasts did not produce CO_2 or grow in processed fruit products containing 0.02% potassium sorbate (a_w 0.89, 55 °Brix) or 0.04% sorbate (a_w 0.93, 45 °Brix). Cole et al. [9] reported that at higher a_w (0.99, 15 °Brix), 0.04% sorbic acid is likely to prevent growth of *Z. bailii* at pH 2.5. These observations do not lend themselves for easy comparison to results from the present study; however, generalizations can be made concerning quantitative methods for predicting growth of food spoilage yeasts. Indirect conductimetry using the automated Malthus instrument is convenient for monitoring yeast activity and generating a large amount of data that can be used to develop predictive models.

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