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Thermal inactivation of two yeast strains heated in a strawberry product: experimental data and kinetic model

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Abstract

The thermal resistances of *Saccharomyces cerevisiae* and *Debaryomyces hansenii* were studied in a high-sugar strawberry product. The effect of the composition of the growth medium on the heat resistance of the two yeast strains was evaluated. The results showed both strains to have different sensitivities to heat, and the cultivation conditions were observed to affect one strain. The decrease in yeast number was not always exponential and survivor curves often presented a preliminary lag phase followed by either an exponential or a parabolic decrease of the surviving population. The results were modelled with a three-parameter formula: $\ln(N_0/mN_t)^a = kt$ where k is the kinetic constant, N_0 and N_t the viable cells at $t=0$ and after heating time t, and m and a are two parameters of the model. m represents the number of cells occurring in clumps and a characterizes the deviation from the exponential decrease. The kinetic constants vary with temperature according to Arrhenius's law. The values obtained in the medium used were higher than those observed in classically studied media like YMPG, particularly when cells were cultivated on malt agar. The results emphasize the need to take account of the non-exponential parts of the survival curves in order to establish thermal process schedules.

Keywords: Fruit products; *Saccharomyces cerevisiae*; *Debaryomyces hansenii*; Thermal resistance

1. Introduction

Pasteurization is an important stage in many processes in the food industry. It contributes not only to the characteristics of the product but also to the destruction of microorganisms which might alter the product during storage and lead to commercial losses. The processed fruit product studied is a high-acid sugared product and its spoilage often comes from the natural microflora of fruits. Therefore, the main spoilage agents are yeasts and moulds. These are relatively heat sensitive [1,2] so that the products generally require only mild treatments. Moreover, to maintain the organoleptic quality of the product it is recommended to keep pasteurization temperatures below 75°C. In the past, microbiologists have focused on the study of the thermal destruction of bacteria. It is only in about the last twenty years that publications have dealt with thermal injury of fungi and have demonstrated that one cannot extrapolate experimental results on bacteria to fungi. Most of those publications report data on the thermal destruction of fungi in synthetic media such as phosphate buffer or sugar solutions [2–4]. These data compared to those obtained for industrial products like fruit juices [1,5,6], wine [7–9] or peach puree [10] show major differences in thermal death rates of the same strain heated in different media. The thermal inactivation rates of yeasts depend particularly on the solutes and their concentrations in the heating medium. Therefore, it is essential to study the thermal destruction of yeasts in the product for which industrial pasteurization schedules have to be established. Moreover, the sensitivity of the yeast is influenced by the composition of the cultivation media.

This report describes an investigation of the thermal injury of *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. These yeasts are among those often involved in the spoilage of fruit-based products [11–13] and are known to grow on high-sugar media [14,15].

The results presented concern the influence of the nature and composition of the culture medium on the thermal inactivation rates. A model was set up to represent non-linear survivor curves and to calculate the corresponding kinetic rates.

2. Materials and methods

2.1. Microorganisms and cultural conditions

Two yeast strains involved in the fruit-based product spoilage were selected for their ability to grow on the medium

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used and for their resistance to industrial pasteurization schedules. They have been identified as *Saccharomyces cerevisiae* and *Debaryomyces hansenii*. They both belong to ascomycetous species and are often reported as being more heat resistant than other yeasts [2,3].

Stock cultures were maintained on malt agar (pH 5.5) at 4°C. Culturing conditions were different according to the phase of the growth medium. The solid medium was the malt agar supplemented with glucose (5 g l^{-1}). Cells were grown for 5 days at 30°C before being suspended in sterile water. The liquid medium had the following composition: glucose (150 g l^{-1}), KH_2PO_4 (5 g l^{-1}), $Mg(SO_4)_2 \cdot 7H_2O$ (0.4 g l^{-1}), yeast extract (1 g l^{-1}), $(NH_4)_2SO_4$ (2 g l^{-1}) and distilled water. The pH was adjusted to 3.8 with orthophosphoric acid. The broth (200 ml) was dispensed in 250-ml Erlenmeyer flasks, sterilized at 120°C for 20 min and inoculated before incubation at 30°C for 24 h for *Saccharomyces cerevisiae* and 36 h for *Debaryomyces hansenii*. Those times were necessary to reach the stationary phase at which the cells are the most heat resistant [16,17]. The yeast suspension consisted of vegetative cells. No sporulation was observed.

2.2. Heating medium and heating experiments

The heat resistances of the yeasts were determined in a sterile, strawberry-based product (48°Brix, $a_w = 0.92$, pH = 3.65). Because of its physical properties and principally its high viscosity, classical methods for the determination of the heat destruction rate [18,19] were not suitable. A modified "thermal death tube" method was therefore adopted. The experiments were performed in 21-ml watertight stainless steel tubes (inner ϕ 22/outer ϕ 25 × 55 mm) closed at each tip with screwed stoppers. Watertightness was ensured by rubber seals. All heating experiments were carried out in a temperature-regulated water bath (Polystat 3386633 of Bioblock Scientist). The experimental procedure was as follows: the heating medium was tempered in different tubes at the test temperature and then inoculated with a yeast suspension so that the inoculation rate was about 5 million cells per millilitre for *Saccharomyces cerevisiae* and 10 million for *Debaryomyces hansenii*. After heating, the tubes were cooled rapidly in an ice/water bath and the contents were transferred into 250-ml Erlenmeyer flasks to enumerate the surviving yeasts. It was checked that the temperature did not decrease while inoculating and that the yeasts received uniform exposure to the target temperature. Experiments were performed at different temperatures from 55 to 70°C according to the strain and culturing conditions. Each experiment was performed in duplicate.

2.3. Enumeration of the viable population

Among four counting methods (pour plate, surface spread, most probable number and ATP measurement) the surface spread method was confirmed to be the most useful for our study [20]. Viable yeasts were enumerated on malt medium plus glucose (5 g l^{-1}) which was selected because it gave relatively large colonies compared with other media. Uninjured cells were visible after 2 days of incubation but additional colonies appeared up to the tenth day of incubation. No further recovery was observed after 11 days of incubation. Plates were incubated at 30°C and colonies were counted at intervals up to 10 days.

2.4. Analysis of results

Each experiment was done in duplicate and for each sample appropriate decimal dilutions were plated on malt agar in triplicate. The method, as presented previously, was reproducible [20]. The experimental results were modelled and the least-squares method was used to calculate the model parameters. The convergence criterion was calculated as follows: crit. $= \Sigma\{(N_{obs} - N_{calc})/N_{obs}\}^2$ where N_{obs} are the experimental data and N_{calc} the values calculated by the model. The correlation coefficient (r^2) was calculated by plotting $\ln(N_o/mN_t)$ as a function of heating time.

3. Results

3.1. Profiles

The thermal inactivation curves of the two selected strains heated in the strawberry preparation are presented in Figs. 1 and 2. Only one of these curves (*Saccharomyces cerevisiae* grown in the liquid medium) is linear when plotted on a semilog scale. All the others have either an initial lag phase before the exponential decrease (*Debaryomyces hansenii* grown in the solid medium) or a parabolic shape (*Saccharomyces cerevisiae* grown in the solid medium or *Debaryomyces hansenii* grown in the liquid medium).

3.2. Models

Several models exist to represent the survival curves (Table 1). For the exponential curves representing the ther-

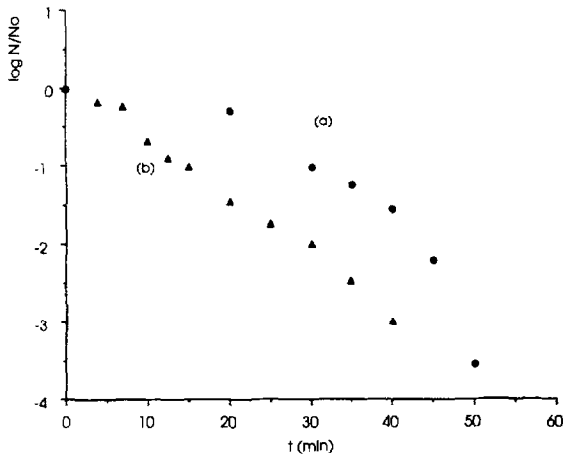

Fig. 1. Survival curves of *Saccharomyces cerevisiae* heated at 61°C in a strawberry product after growth on: (a) malt agar; (b) the liquid medium.

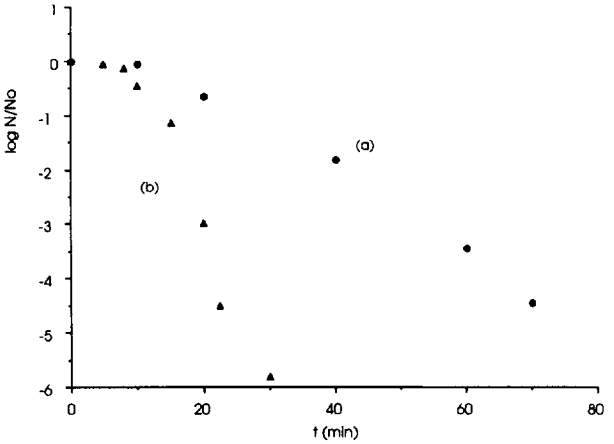

Fig. 2. Survival curves of *Debaryomyces hansenii* heated at 58°C in a strawberry product after growth on (a) malt agar; (b) the liquid medium.

mal inactivation of *Saccharomyces cerevisiae* cultivated in a liquid medium, the kinetic rates were calculated by the classical Chick's law [21]:

$$\ln\frac{N_o}{N_t}=kt \tag{1}$$

Survivor curves of *Debaryomyces hansenii* grown in the liquid medium and *Saccharomyces cerevisiae* on the solid medium have the same profiles. They are similar to those obtained by Douglas King et al. [22] for the mould *Byssoclamys fulva* heated in a 5°Brix product. These survivor curves were well represented by the formula:

$$\left[\ln\frac{N_o}{N_t}\right]^a=kt \tag{2}$$

where k is the kinetic constant and a a parameter which characterizes the deviation from the exponential decrease. The *Debaryomyces* strain heated in the strawberry preparation after growth on malt agar exhibits different behaviour from the other three. Its survivor curve begins with an initial lag phase and is then exponential. In this case the phenomenon can be modelled by:

$$N_t=N_o-N_o(1-\exp(-kt))^m \tag{3}$$

where k is the kinetic constant and m a parameter introducing the concept of cell clumping. For each clump and as long as cells of this clump are not all destroyed, we will count only one colony forming unit (CFU) on the plate, which explains the "lag phase". After the destruction of the clumps, the kinetics are exponential.

Finally, a single formula could represent all the survivor curves that we obtained:

$$\left[\ln\frac{N_o}{mN_t}\right]^a=kt \tag{4}$$

The three parameters involved in these models were identified by the least-squares method. Table 2 reports the values of a and m. They did not vary with temperature. The cell suspension of *Debaryomyces hansenii* cultivated on malt agar showed clumps. The calculated m value can be considered as the average number of cells per clump observed under a microscope.

3.3. Thermal death rate

The kinetic constants k of each strain and conditions of culture are given in Tables 3–6 for different temperatures.

Table 1
Literature models to represent the survival curves of microorganisms

Authors	Curve shape	Model	Assumptions
Chick [21]	Exponential	$-\frac{dN}{dt}=kN;\ \ln\frac{N_t}{N_o}=kt$	
Han (1976) quoted by Barillère et al. [8]	Parabolic	$\ln\frac{N_t}{N_o}=-k_o t+\frac{\sigma}{2}t^2$	Heterogeneous population
Han (1976) quoted by Barillère et al. [8]	Parabolic	$\ln\frac{N_t}{N_o}=k_o(1-\alpha)t-\alpha\beta\left(\exp\left(-\frac{t}{\beta}-1\right)\right)$	Heterogeneous population
Van Uden quoted by Deveze [8]	Exponential with an initial lag phase	$\frac{N_t}{N_o}=1-(1-\exp(-kt))^m$	Clump of cells
Douglas King et al. [22]	Parabolic	$\left(\log\frac{N_t}{N_o}\right)^a=-kt+c$	
Barillère et al. [8]	Exponential with an initial lag phase	$\ln\frac{N_t}{N_o}=-kt+\ln m$	Flocculation
Bailley and Ollis [23]	"Retardant law"	$\ln\frac{N_t}{N_o}=-\frac{1}{a}k\ln(1+at)$	
Bailley and Ollis [23]	"Logistic law"	$-\frac{dN}{dt}=kN+k'N(N_o-N)$ $\ln\left(\frac{kN_t}{N_o(k+k'(N_o-N_t))}\right)=(k+k'N_o)t$	

Table 2
Parameter values in Eq. (4) for the thermal destruction of *Saccharomyces cerevisiae* and *Debaryomyces hansenii* heated in a strawberry-based product for differents conditions of culture

Strain	Growth medium	a	m
S. cerevisiae	Liquid	1	1
S. cerevisiae	Solid	0.478	1
D. hansenii	Liquid	0.486	1
D. hansenii	Solid	1	$1 < m < 10$

Table 3
Saccharomyces cerevisiae cultivated in the liquid medium: $\ln\frac{N_t}{N_o} = kt$

Temperature/°C	k/min^{-1}	D/min	r^2
60	0.11	21.3	0.95
62	0.17	13.5	0.99
63	0.29	7.9	0.98
64	0.41	5.6	0.97
65	0.63	3.6	0.9
68	2.21	1.	0.97
70	7.91	0.29	0.99

Table 4
Saccharomyces cerevisiae cultivated on malt agar: $\left(\ln\frac{N_o}{N_t}\right)^{0.479} = kt$

Temperature/°C	k/min^{-1}	r^2
61	0.031	0.96
63	0.062	0.968
65	0.12	0.921
67	0.24	0.99
70	0.67	0.97

Table 5
Debaryomyces hansenii cultivated in the liquid medium: $\left(\ln\frac{N_o}{N_t}\right)^{0.486} = kt$

Temperature/°C	k/min^{-1}	r^2
55	0.06	0.996
58	0.094	0.972
60	0.20	0.984
62	0.33	0.958
65	0.68	0.84
67	1.12	0.885

Table 6
Debaryomyces hansenii cultivated on malt agar: $\frac{N_t}{N_o} = 1 - (1 - \exp(-kt))^m$

Temperature/°C	k/min^{-1}	m	Crit.
55	0.034	1.62	0.049
57	0.093	6.85	0.032
58	0.173	9.61	0.09

The severity of the heating treatment may have masked the real shape of the curve. This can explain why the correlation coefficients are not so good for the higher temperatures. Arrhenius's law was applied to represent the evolution of k with the temperature:

$$k = A \exp\left(\frac{-E_a}{RT}\right) \quad (5)$$

where T is the temperature in Kelvin. The values of the parameters E_a and A are reported in Table 7. The temperature

Table 7
Variation of kinetic rate with temperature: $k = A \exp\left(\frac{-E_a}{RT}\right)$

	S.cerevisiae (liquid)	*S. cerevisiae* (solid)	*D. hansenii* (liquid)	*D. hansenii* (solid)
E_a/kJ mol^{-1}	406.6	326.46	230.62	483.59
ln A	144.5	114.14	81.74	174
r^2	0.985	0.94	0.989	0.996

Table 8
Values of the "z" parameter: $\log\frac{(2.303)^a}{k} = -\frac{T}{z} + c$

	S. cerevisiae (liquid)	*S. cerevisiae* (solid)	*D. hansenii* (liquid)	*D. hansenii* (solid)
z/°C	5.36	6.72	9.11	4.29
r^2	0.987	0.999	0.996	0.996

elevation necessary to obtain a kinetic constant 10 times higher was also calculated. This parameter, often called "z", is related to the decimal reduction time. To keep close to this concept, we applied the formula:

$$\log\frac{(2.303)^a}{k} = -\frac{T}{z} + c \quad (6)$$

with T expressed in °C. z-values are listed in Table 8.

4. Discussion

4.1. Profiles

Several authors have previously observed deviations from the exponential decrease. Barillère et al. [7] obtained different profiles according to the yeast species. They reported bilinear curves for a *Saccharomyces cerevisiae* strain, curvilinear for *Saccharomyces bailii* and S"shaped curves for *Saccharomyces pombe* and *Saccharomyces bayanus* grown and heated under the same conditions. Juven et al. [1] noted that the number of viable cells in the fruit juices or model solutions they tested did not decrease exponentially with the time of exposure to heat, especially in the range 50–60°C. In our case the phenomenon persisted beyond 60°C up to 70°C. For higher heating temperatures, the curves may appear exponential because of the high rates of inactivation. In those case the severity of the treatment may mask the curvilinear portion of the survivor curves. Several assumptions have been made to explain these deviations. According to Barillère et al. [8] populations of vegetative cells are more heterogeneous than those of spores, which may lead to parabolic curves rather than exponential. The deviations have also been attributed to heterogeneous populations, cell clumps or flocculation. Furthermore, the enumeration method was sometimes incriminated, particularly in the case of tailing curves. Our experiments prove that a difference occurs when a strain is cultivated under different conditions even when heated in the same medium. Our purpose is not to try to interpret this phenomenon but to suggest empirical models to fit our results in order to calculate thermal death rates and pasteurization schedules.

4.2. Model

Generally, when non-exponential curves are encountered, authors either neglect the lag phase or the unusual shape and apply the classical Chick's law to determine the decimal reduction time, or they do not give kinetic rates and analyse their results only from a qualitative point of view. It seems to us that the classical way to calculate the thermal death rate is not always suitable for establishing pasteurization schedules. In some cases it will be important to take the lag phase into account.

Indeed, our results show that the first log unit of destruction of *Debaryomyces hansenii* grown on agar medium takes 25 min at 58°C, whereas the following units take only 13 min, and a total of 6 log units of destruction takes 95 min. With regard to the *Saccharomyces* strain grown on malt agar and heated at 61°C, the first log unit of destruction lasts 30 min, the second only 15 min and a 6 log unit kill takes 90 min. In these two examples the first unit of destruction represents the major part of the thermal destruction time. Therefore, it would not be rigorous to use only the linear part of the curves to calculate process schedules. This could explain why some yeasts resist pasteurization processes.

4.3. Thermal death rates

From our results, it appears that the two strains do not react in the same way when the temperature increases. *Saccharomyces cerevisiae* seems to be more sensitive to the elevated temperatures when grown in a liquid medium. On the contrary, the thermal destruction of *Debaryomyces hansenii* takes less time when the cells are cultivated on a solid medium. These results emphasize the different behaviour which exists between the different yeast strains with regard to thermal destruction. Figs. 1 and 2 as well as Tables 2–6 indicate that not only the profile but also the thermal death rate changes with the strain and its culture conditions. In both case, the

Table 9
Bibliographic data on the thermal destruction of *Saccharomyces cerevisiae* heated in different media

Authors	Heating medium	Results	
Hernandez and Feria [25]	Strawberry jam (65°B)	Thermal death time at 80°C (510^4 cells per g of jam)	14 min $z = 11.7$°C
Juven et al. [1]	Orange juice (50°B) Sucrose/glucose solution (50°B)	Percentage of the surviving population after 8 min at 60°C	79% 3%
Juven et al. [1]	Sucrose solution (48%)	Decimal reduction time at 65°C	1.7 min
Splittoesser et al. [6]	Apple juice (8.6°B) (18.5°B)	Decimal reduction time at 60°C	6.1 min 12 min
Splittoesser et al. [6]	Aqueous solution Sugar solution (10%)	Decimal reduction time at 60°C	2.4 min 4.6 min

cells are more resistant after growth on malt agar medium but a quantitative comparison is difficult since the shapes of the survivor curves are different. The thermal resistances obtained in the four cases are higher than those reported in the literature. The complexity of the heating medium and its high sugar concentration can explain this difference. Gibson [24] and Stecchini and Beuchat [10] demonstrated that sucrose was a metabolizable sugar that led to the slower inactivation rates. These authors attributed this effect to dehydration together with a reduction in the pore size of the cell membrane. To make a comparison with these data, we have reported different bibliographic data related to a *Saccharomyces cerevisiae* strain heated in different media (Table 9). The resistance values are much higher than those presented in the literature for sucrose solutions even at the same Brix. This enhances the error that may occur when pasteurization schedules are calculated using results obtained on synthetic media. Very few publications have dealt with the thermal destruction of yeasts in processed fruit products. The most we can find is on fruit juices. The values we obtained are higher than those obtained by Splittoesser et al. [6] on an apple juice. This can be explained by the difference between the two product Brix (48°B versus 18°B). Our experimental data are close to those of Juven et al. [1]. Unfortunately, those authors do not suggest any inactivation rate constants.

Our experimental results illustrate that thermal destruction kinetics vary according to the strain and the conditions of culture. The study confirms that *Saccharomyces cerevisiae* is one of the most heat-resistant strains even in a complex medium and, therefore, should be used to test any process schedule as far as low-pH sugared media are concerned. We found not only different profiles but also different kinetic rates. Because of the non-logarithmic kinetics, the classical way to establish thermal process schedules is not always suitable. Cell clumps as well as special growth conditions may explain the resistance of yeasts to industrial pasteurization schedules. Therefore, we recommend that an extensive study of the heat destruction of yeasts is undertaken directly in the industrial product. Attempts should be made to determine the culture conditions that lead to the maximal heat resistance of the yeast in the product and to know the profile of the kinetics before developing thermal process schedules.

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