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PHYSICOCHEMICAL DIFFERENCES IN DEHYDRATED *SACCHAROMYCES BOULARDII* YEAST AS A FUNCTION OF THE DEHYDRATION PROCESS

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Abstract

Measurements of the masses of incorporated water and of the heats of hydration have been performed at 310 K, under air atmosphere saturated with water vapour, on three differently dehydrated samples of the *Saccharomyces boulardii* yeast in order to evaluate differences in physicochemical properties related to the following dehydration processes: cryodesiccation (or lyophilization) and thermodesiccation (spray drying and in-fluidized-bed heating).

Thermogravimetric (TG) experiments have shown that the lyophilized yeast contains less residual water than heat-treated yeasts and that it incorporates more water and more rapidly. Isothermal water sorption of a spherical yeast cell has been represented by a two-parameter equation able to take account of a maximum of the hydration rate when this is taken as a function of time. On the other hand, isothermal DSC experiments have shown that hydration-heat values are higher for the heat-treated yeasts than for the lyophilized one. The TG and DSC results have been shown to be consistent with the idea that the physicochemical properties of a dehydrated yeast are related to cell-wall behaviour during desiccation.

Keywords: hydration heats, isothermal hydration, lyophilization, *Saccharomyces boulardii* yeasts, thermodesiccation

Introduction

Saccharomyces boulardii is a non toxic yeast first isolated by Boulard from the lychee fruit in Indochina. It is an ellipsoidal, oval or sometimes spherical cell whose wall has a polysaccharide structure, whereas the cytoplasm contains aminoacids, B vitamins, enzymes and certainly many other compounds. Since the fifties this yeast has been used for the treatment of infectious [1], antibiotic-induced [2, 3] and bacterial-associated [4, 5] gastrointestinal illnesses. Moreover, some inhibition of the effects of bacterial toxins, such as that of water secretion induced by prior inoculation of cholera toxin, has been pointed out [6, 7]. It has also been found to have a

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protective effect if challenged with either toxigenic Clostridium difficile or purified toxin A or B [1].

For pharmaceutical purposes related to oral administration (capsule dosing and storage), cultures of the yeast have to be dehydrated into a powder. Two dehydration techniques, both founded upon the tendency of water to vaporize, are commonly employed: cryodesiccation (lyophilization or freeze-drying) and thermodesiccation (spray-drying and fluidized-bed heating). Lyophilization should be largely preferable since it is the only one which ensures the prolonged preservation of microorganisms.

Dried cells can be revived on any appropriate medium. In particular, a rapid rate of vitality recovery, when yeast is delivered in the bowel, is thought to depend on the rate of the in vivo hydration process. In order to evaluate the physicochemical differences among otherwise dehydrated yeast samples, thermogravimetric and calorimetric measurements of the incorporated water mass and of the hydration heat, respectively, were performed at 310 K under air atmosphere saturated with water vapor.

Experimental

Thermogravimetric measurements of the mass loss as a function of increasing temperature were made on lyophilized, spray-dried and fluidized-bed heated yeasts in order to determine the residual water content (mg per 100 mg of yeast) after drying processes. The three samples resulted from treatment of two batches (K 242 and J 253) of the same strain *Saccharomyces boulardii*. Reproducible production of cultures of this strain by Biocodex Laboratories is well established and validated. Experimental conditions for manufacture of samples are hereafter given. Lyophilized sample: the suspension of the yeast (batch n° K 242) is quickly frozen at –28°C and then sublimed by heating at 25°C during 24 h. Spray-dried sample: spraying of a yeast suspension (yeast batch n° J 253), entering air at 140°C, leaving air at 110°C for a process of 25 min. Fluidized-bed heated sample: pulverisation of a yeast suspension (yeast batch n° K 242), entering air at 100°C, leaving air at 70°C for a process whose duration is 20 min. Samples were stored in glass vessels closed inside a glove box under He atmosphere (O_2<1 ppm, H_2O<1 ppm) which were open just before performing measurements.

Scans at the heating rate of 10 K min^{-1} under a nitrogen flux were obtained by means of a TA Instruments (USA) TGA 50 thermogravimetric analyzer. The same apparatus was implemented to perform isothermal hydration measurements under air atmosphere saturated with water vapor. In this case, an empty container was placed near the suspended sample in a quartz tube provided with an open end and situated inside the thermobalance furnace. The sample chamber was first heated up to the chosen temperature; then water, previously kept at the programmed temperature, was injected into the container by means of a syringe, and the open end of the furnace tube immediately sealed.

Isothermal measurements of the hydration heats were performed by means of a TA Instruments DSC 10 differential scanning calorimeter. Samples (around 10 mg) were placed into open aluminum pans and weighed before being introduced in the DSC cell. A perforated plastic vessel was overturned and fitted to the top of the cell.

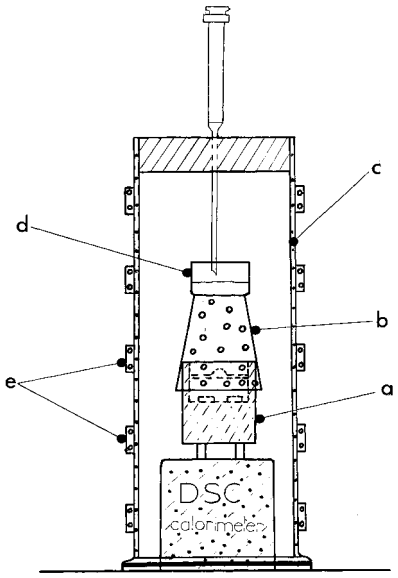

Fig. 1 Design of the home-adapted DSC calorimeter for isothermal hydration-heat measurements; a – calorimetric head; b – overturned perforated vessel; c – cylindrical jar; d – beaker; e – ribbon resistance

A glass beaker was glued at the upward base of the vessel in order to be filled with distilled water. Around the DSC cell, a glass cylindrical jar was installed whose top exhibited an orifice conceived for injecting water into the beaker by means of a syringe. A heating ribbon was carefully coiled around the outer walls of the jar in order to equalize the temperature of the dead volume inside the jar to that of the DSC cell during hydration processes and to reduce to a minimum the 'cold wall' effects. In a hydration measurement this home-conceived apparatus (Fig. 1) was first heated up by means of the ribbon resistance only. When the DSC-cell temperature was stable and 0.3 K lower than the programmed temperature the DSC resistance started with heating. Water was injected once the constant programmed temperature was attained. Samples were rapidly weighed at the end of the experiments for the amount of incorporated water to be determined.

Results and discussion

Thermogravimetric studies

Temperature scanning

In Fig. 2 the TG curves of the three samples of dehydrated yeast are presented. One can notice that mass losses occurred in two steps. The first one was attributed to a dehydration process. By opening the punctured sample pans after cooling we realised independent of the treatment of the yeast, that the second step reflected a ther-

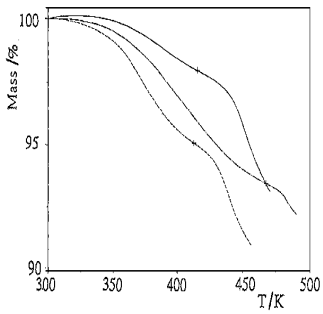

Fig. 2 Temperature-scan TG traces of dehydrated yeasts. Curves; solid: lyophilized yeast; dashed-dotted: fluidized-bed heated yeast; dashed: spray-dried yeast. In all cases, the cross points on curves indicate the end of the thermal dehydration

mal degradation. The end of the dehydration was estimated to correspond to the temperature, between the two steps of mass losses, for which the derivative function of the mass with respect to temperature exhibited a relative minimum. It can be noticed in Fig. 2 that for lyophilized and spray-dried yeasts these temperatures were lower than that recorded for the fluidized-bed heated one. Thus, we could argue that lyophilized and spray-dried yeasts dehydrates more readily than fluidized-bed heated ones.

From these results it turned out that the freeze-dried yeasts contained less residual water than the heat-treated ones.

Isothermal mode

In Fig. 3 the hydration curves of three different samples of each type of yeast are illustrated. In Table 1 the values of mass of incorporated water, of highest rate of incorporation and of elapsed time for attaining the maximum rate are reported. It may be remarked that, under atmosphere saturated with water vapour, lyophilized yeasts incorporated more water and more rapidly than the heat-treated yeasts. The traces in Fig. 3 exhibited a sigmoidal feature, independent of the yeast. This indicated that hydration processes were kinetically characterized by induction periods (the above de-

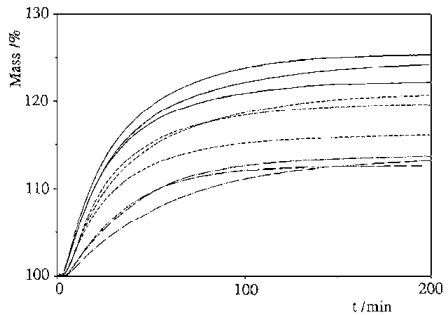

Fig. 3 Traces of the TG isothermal measurements (310 K) of the yeast water uptakes. Curves; solid: lyophilized yeasts; dashed: spray-dried yeasts; dashed-dotted: fluidized-bed heated yeasts

Table 1 Physicochemical data on water sorption of three dehydrated yeasts. The highest rates for water uptake are given, also in the following table, as μg (water) min^{-1} mg $(yeast)^{-1}$

Yeast	Lyophilized	Spray-dried	Fluidized-bed heated
Residual water content/mg (100 mg of yeast)$^{-1}$	2.2	5.0	6.6
Water uptake in mg for 100 mg of yeast	24.3	19.4	13.6
	25.2	16.4	13.7
	22.2	20.7	12.7
Uptake highest rate in μg min^{-1} mg^{-1}	6.8	6.0	2.2
	7.4	5.1	3.0
	6.8	5.3	3.1
Elapsed time for the highest rate to be reached in min	4.3	8.3	6.7
	3.9	6.7	7.6
	5.5	7.8	7.5

fined elapsed times) which were clearly shorter for the lyophilized samples than for the other ones. The same traces showed also that hydration evolved toward a saturation limit of incorporated water, which was practically attained after 200 min. It is likely that the induction periods correspond to times necessary to make the cell wall permeable and, consequently, to modify its properties.

On the other hand, if one considers that the yeast cell has a spherical shape, the advancement of the hydration front inside the sphere may be described by the phenomenological equation:

$$\alpha = 1 - \exp(-kt^n) \tag{1}$$

where α is the advancement degree of the hydration, t is time and k and n are experimental parameters. This equation has a form identical to those proposed by Johnson and Mehl [8] and by Avrami [9–11] to describe the nucleation of a phase β during a phase transformation α→β.The main feature of this equation is that it can be forced to take account of the initial induction period at the end of which the hydration rate exhibits a maximum. In fact, its derivative is:

$$\frac{d\alpha}{dt} = knt^{n-1}\exp(-kt^n) \tag{2}$$

which has a maximum real value for:

$$t = \left(\frac{n-1}{nk}\right)^{0.5} \tag{3}$$

in the case n>1 and k>0. For this reason it has been chosen to describe in an easy way a complex phenomenon such as the first step of the yeast cell hydration.

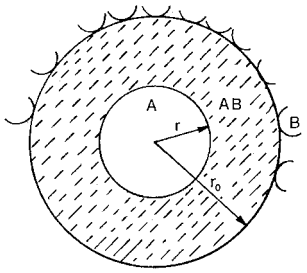

Fig. 4 *Saccharomyces boulardii* cell as pictured during hydration. A – non-hydrated portion of the cell at time *t*; AB – hydrated portion; B – water molecules; r_o – sphere radius at the beginning of hydration (t=0); r – radius of the non-hydrated part of the cell at time *t*

If one considers a single cell before hydration to be a sphere of radius r_o, r ($r< r_o$) being the radius of the inner yeast sphere not hydrated at time *t* (Fig. 4), it follows that:

$$1 - \alpha = \left(\frac{r}{r_o}\right)^3 \tag{4}$$

By substituting in Eq. (2) the values of α and $1-\alpha$ expressed as functions of the ratio $(r/r_o)^3$ we can derive the following differential equation:

$$-\frac{dr}{dt} = \frac{1}{3}knrt^{n-1} \tag{5}$$

Equation (5) shows that the rate of advancement of the hydration front is zero at t=0, for which r=r_o, and for t=t_f, when r=0, respectively, that is to say, at the beginning and at the end of hydration, when the cell is fully hydrated. The final time t_f must theoretically be equal to infinity. As a matter of fact, by substituting Eq. (3) into Eq. (1) or by integrating Eq. (4) after separation of the variables r and t we obtain:

$$r = r_o \exp\left(\frac{-kt^n}{3}\right) \tag{6}$$

In Table 2 we have reported, for three samples of each type of dehydrated yeast obtained from the same *Saccharomyces boulardii* strain, the values of the maximum hydration rates $m_{wt}(d\alpha/dt)_{max}/m_{ys}$ calculated by means of Eqs (1), (2) and (3) (m_{wt} is the mass of incorporated water, $(d\alpha/dt)_{max}$, the hydration rate at time t=$[(n\text{-}1)/(nk)]^{0.5}$ and m_{ys} the mass of the dehydrated yeast), as well as the related values of elapsed times. According to experimental observations, the lyophilized samples are found to exhibit the higher hydration rates and the smaller elapsed times. In order to better represent the initial induction period and, consequently, obtain the most significant properties of each yeast undergoing hydration, the k and n values of Eq. (1) were obtained by interpolating data recorded during the first twenty minutes. As a consequence of this choice and of the fact that introducing any further parameters without

Table 2 Calculated physicochemical data on water sorption of three dehydrated yeasts. Parameters n and k have been obtained by linear interpolation of experimental data on hydration advancement degree (α) as a function of time (t) by means of the logarithmic form of Eq. (1): $\ln|\ln(1-\alpha)|=\ln k+n\ln t$. As in Table 1, for each kind of yeast, the results (three) reported in the numerical cases are given in the order according to the sample numbering. For example, for the lyophilized yeast, the n and k values to be referred to for the first investigated sample are n=1.1574 and k=0.0021

Yeast	Lyophilized	Spray-dried	Fludized-bed heated
$10^4 n$	11570	14590	13110
	11690	13130	12210
	11960	13660	12470
$10^4 k$	210	92	70
	219	146	121
	203	99	137
Uptake highest rate in	6.6	5.8	2.3
$\mu g\ min^{-1}\ mg^{-1}$	6.5	5.1	2.8
	6.4	5.3	3.0
Elapsed time for the	5.0	11.2	14.7
highest rate to be reached in min	4.6	8.4	9.2
	5.7	11.2	8.5

physical meaning, calculated α-data for the final hydration periods may be up to 15% higher than the measured ones.

The rough representation by Eq. (1) of the final part of the hydration process has to be ascribed to the lack of a term taking into account the hydration delay. This time lag could be related to the conceivable dependence of the diffusion coefficient of water on the thickness and on the nature of the hydrated yeast layer $r_o-r(t)$, when it increases.

Calorimetric study

Hydration of yeasts is accompanied by an exothermic heat change. Since areas of the heat of hydration are related to the total mass of incorporated water, the advancement degree of the hydration process could be determined at each time t by calculating the ratio of the area of the peak fraction at time t to the whole peak area. This would offer an attractive alternative for cases where thermogravimetry cannot be implemented. In this work, values of the hydration heats correspond to average values of at least three measurements at 310 K. The hydration heats for lyophilized, spray-dried and fluidized-bed heated yeasts were determined to be 32.6±0.9, 41.1±0.6 and 38.5±1.3 kJ $mol^{-1}$of incorporated water, respectively. These results are in fair agree-

ment with the conclusions drawn from T-scan thermogravimetric measurements that the freeze-dried yeast lost residual water at a lower temperature than the fluidized-bed heated yeast. Although the hydration heat of lyophilized yeast is lower than those of the heat-treated yeasts, the water uptake is faster. In fact, peak maxima, which correspond to the highest hydration rates, occurred after elapsed times varying between 6 and 7.5 min for the freeze-dried yeast, whereas they occurred after 12 to 15 min and after 14 to 23 min for the spray-dried and fluidized-bed heated yeasts, respectively. A synoptic way to show that lyophilized yeast hydrates faster than the other ones consists in superimposing the related peaks.

In Fig. 5a and 5b the hydration peak of a lyophilized yeast has been compared to those of the other kinds of yeast. In both cases, the heat associated to the hydration of the freeze-dried yeast remains higher than those inherent to the other two types up

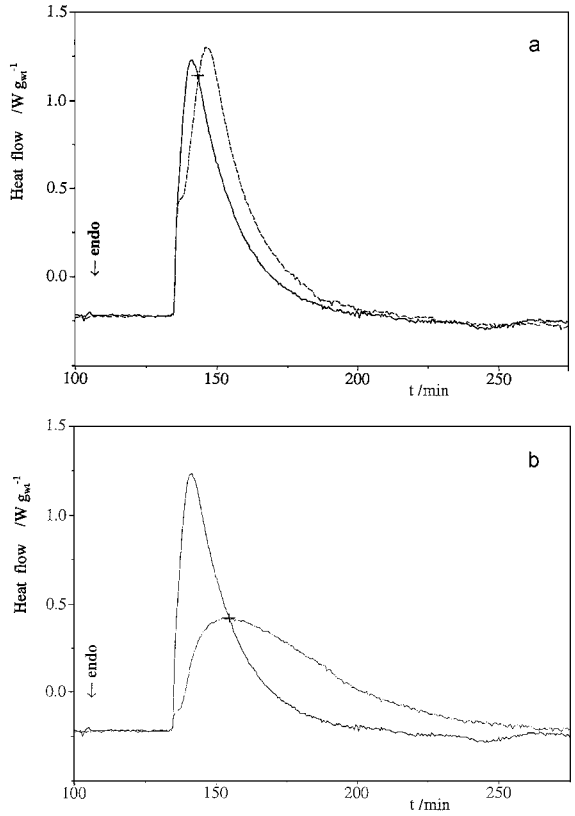

Fig. 5 Isothermal hydration-heat DSC measurements at 310 K. Superimposed hydration peaks: a) the peak of a lyophilized yeast (solid curve) to that of a spray-dried one (dashed curve); b) the same lyophilized-yeast peak to that of a fluidized-bed heated yeast (dashed-dotted curve). In both cases, the differences between times corresponding to cross-point abscissae and initial times coincide with the beginning hydration periods during which the related heats turn out to be higher for the lyophilized yeasts than for the other two kinds of yeasts

to times (corresponding to the abscissae of the cross points on Fig. 5a and 5b) longer than those of its maximum hydration rate. The fact that the heat for hydration of the lyophilized yeast is smaller than those for hydration of the heat-treated yeasts is probably related to the different behaviour of the cell walls during desiccation. It has been observed that cell walls remain porous after being lyophilized while become less permeable after being heat-treated, presumably because of chemical or conformational changes. If it is assumed that these changes occur during thermal dehydration and are endothermic and reversible, the converse ones could occur during hydration and would be responsible for the more exothermic peaks recorded for the heat-treated yeasts. Further work will be necessary in the future to elucidate the physicochemical aspects related to a dehydration-hydration cycle on a yeast.

Conclusions

This work shows that *Saccharomyces boulardii* yeast powders obtained by lyophilization and by heat treatment differ from each other with respect to macroscopic physicochemical properties. The lyophilized yeast contains less residual water and incorporates more water than the heat-treated yeasts under air atmosphere saturated with water vapor. These differences could be related to the behaviour of the cell walls during desiccation: they remain porous after lyophilization while become less permeable after heat treatment, probably because of chemical or conformational changes occurring in these living systems. This explanation seems to be corroborated by isothermal measurements of the hydration heat by DSC at 310 K. The hydration heat values are distinctly higher for the heat-treated yeasts than for the lyophilized one.

References

1 L. V. McFarland and P. Bernasconi, Microbiol. Ecol. Health Dis., 6 (1993) 157.
2 R. D. Toothaker and G. W. Elmer, Antimicrob. Agents Chemother., 26 (1984) 552.
3 C. M. Surawicz, G. W. Elmer, P. Speelman, L. V. McFarland, J. Chinn and G. Van Belle, Gastroenterology, 96 (1989) 981.
4 J. P. Buts, G. Corthier and M. Delmee, J. Pediatr. Gastroenterol. Nutr., 16 (1993) 419.
5 C. M. Surawicz, L. V. McFarland, G. W. Elmer and J. Chinn, Am. J. Gastroenterol., 84 (1989) 1285.
6 N. Vidon, B. Huchet and J.-C. Rambaud, Gastroenterol. Clin. Biol., 10 (1986) 13.
7 D. Czerucka, I. Roux, J. L. Nano, P. Bernasconi and P. Rampal, Gastroenter. Clin. Biol., 13 (1989) 383.
8 W. A. Johnson and R. F. Mehl, Trans. Am. Inst. Mining Met. Engrs., 135 (1939) 416.
9 M. Avrami, J. Chem. Phys., 7 (1939) 1103.
10 M. Avrami, J. Chem. Phys., 8 (1940) 212.
11 M. Avrami, J. Chem. Phys., 9 (1941) 177.