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VIABILITY OF YEAST *SACCHAROMYCES CEREVISIAE* DRIED IN THE PRESENCE OF LACTOSE[*]

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Abstract. The influence of lactose addition on viability of yeast *Saccharomyces cerevisiae* dried using freeze-drying and microwave-vacuum drying methods was investigated. Lactose was co-immobilized with yeast suspension in Ca-alginate matrix in the form of beads and than dried. It was found that addition of sugar can improve viability of cells during freeze-drying but not during microwave-vacuum drying.

Keywords: freeze-drying, microwave-vacuum drying, lactose, yeasts

INTRODUCTION

Various techniques are used for the dehydrating of thermolabile biological materials, starting with separation as a result of filtration and centrifugal separation of suspensions, ending with complicated drying methods at liquid and solid states in which the movement of moisture is evoked by temperature or pressure gradients.

Lyophilization is the method most often used in biotechnology (Bednarski 1990). The application of reduced pressure allows to evaporate ice from a cell at room temperature; the biomaterial being dried retains its structure and porosity, and after rehydration, a high degree of the enzymes is retained (Cerrutti *et al.* 2000). That method is used to fix proteins and enzymes and viable cells in the collection of microorganisms (Bekatarou *et al.* 2001). An obstacle in using lyophilization for the fixation of industrial micro-organisms is still high cost of the

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system to be used for the freeze-drying and the need to match the drying process parameters to the strain being dried, this being associated with the performance of a thorough analysis of physicochemical, morphological and biotechnological properties during the drying and storage.

A drying method referred to as guaranteeing a high conservative capacity of thermolabile compounds is drying at microwave heating (Piotrowski *et al.* 2005). A characteristic feature distinguishing this drying method from other methods is that the temperature inside the material can be higher than on the surface, whilst the moisture from the material interior flows in the direction of the surface due to thermal diffusion. A method to remove the water collecting over the sample is the use of vacuum in the drying chamber as a result of which the steam flow moves towards the condenser and the pump on the basis of pressure differential. Basing on the positive results of experiments in drying medicines and vegetables and pharmaceutical herbs containing very unstable aromatic compounds (Drouzas *et al*. 1996, Loughlin *et al.* 2002, Szarycz *et al.* 2002, Baillon *et al.* 1996), a presumption may be raised about potential for the application of that drying method for the preservation of micro-organisms.

During the dehydration process the original fixed status of the cell is infringed, and while striving for reducing the moisture contents to a level where both the organism and the environment are in a thermodynamic balance, the system is introduced into a new fixed status (Chmiel 1998). The organism loses the free, biologically active water, and the bound water remains therein, having the form of polar group areolas constituting a skeleton of the protoplasm gel. In the bound water, substances found in water spaces of the gel structure are not diluted and this is one of the reasons for the stoppage of biochemical transformations. The transformation of protoplasm proteins from hydrosol into hydrogel, progressing during the rehydration of the cell, leads to a stoppage of vital functions of the systems, to a degree that is dependent on the amount of water (Kudra *et al*. 1998). In the new fixed status – anabiosis, all the vital processes continue to a minimum degree, and in the event of occurrence of proper conditions their intensity rises to a normal level (Nicklin *et al*. 2004). In reality, the anabiosis produces, within a part of population, a lethal effect the reason of which may be, among others, the infringement of the cell membrane (destruction, deformation) or the denaturation of cell protein (Bayrock *et al*. 1997). An advantageous effect of the improvement in the survival rate of the biomass being fixed can be obtained using an additive of membrane-active adjustment factors such as: trehalose, lactose, sucrose, glucose, hydrocolloids, honey, whey or skim milk (Abadias *et al*. 2001) or the immobilization of cells on a porous carrier (Turker *et al*. 1998).

The aim of this paper was to determine the impact of an additive of lactose on the physiological condition of yeast cells being immobilized in calcium alginate during the freeze and microwave-vacuum drying processes.

MATERIAL AND METHODS

Yeast *Saccharomyces cerevisiae* S.ca./14, purchased from the Collection of Industrial Micro-organisms of the Institute of Agricultural and Food Biotechnology in Warsaw was used. *Saccharomyces cerevisiae* was grown in 300 ml shake flasks containing 100 ml pre-culture medium containing 0.3% yeast extract, 0.3% malt extract, 0.5% peptone and 1.0% glucose. The medium was adjusted to pH 5.0 and sterilized at 121^{o}C for 20 min. The pre-culture was cultivated at 30^{o}C for 24 h. Exponential phase cells (more than 1 x 10^{8} cell ml^{-1}) were harvested by centrifugation (3000rpm, 5min) and washed twice with sterile distilled water (Isono *et al*. 1995).

A mixture of 3% alginate gel (with a 2% additive of lactose or without additive) and a standardized inoculum was instilled to 0.2 M of sodium chloride solution. The microencapsulating was made using a multi-needle syringe at room temperature while stirring the chloride with a magnetic stirrer. Yeast concentration in the mixture was 0.01 g_{D100} ml^{-1}.

The freeze-drying was conducted in a drier type OES 950 (Kramkowski *et al*. 2000), distributing the filtered-off alginate-yeast beads on stainless steel trays and using the freezing down to –40^{o}C and afterwards, the ice sublimation at the temperature of the heat plate at a level of 30^{0}C and a pressure of 100 Pa.

The microwave-vacuum drying was performed in a drier type SM-200 (Szarycz *et al*. 2002), using variable pressure within a range between the pressure of p_A = 6 kPa, at which the vacuum pump was turned on and p_B = 4 kPa, at which the pump was turned off. The microwave power was set at the level of 360W.

Rehydration of dried material was carried out in sterile distilled water at 30^{o}C for 30 min. Micro-beads were than dissolved in 0.2M Na-citrate in shake flasks, after which the entrapped cells were sampled.

The survival rate of yeast was assessed basing on the analysis of the physiological condition of cells as seen in the microscope preparation after dyeing with methylene blue (dead cells are dyed blue) (Nedović *et al*. 2001) in relation to the number of live cells before the drying process.

The water activity (meter type KMAW 7 made by COBRABID) and moisture contents (per PN-73/A-79005) were determined. The determinations were done at 10-minute intervals for the microwave-vacuum drying and at 60-minute intervals for sublimation drying.

RESULTS

Freeze-drying

The viability of yeast populations being lyophilised, in the function of water activity and moisture contents, is presented in Figures 1 and 2.

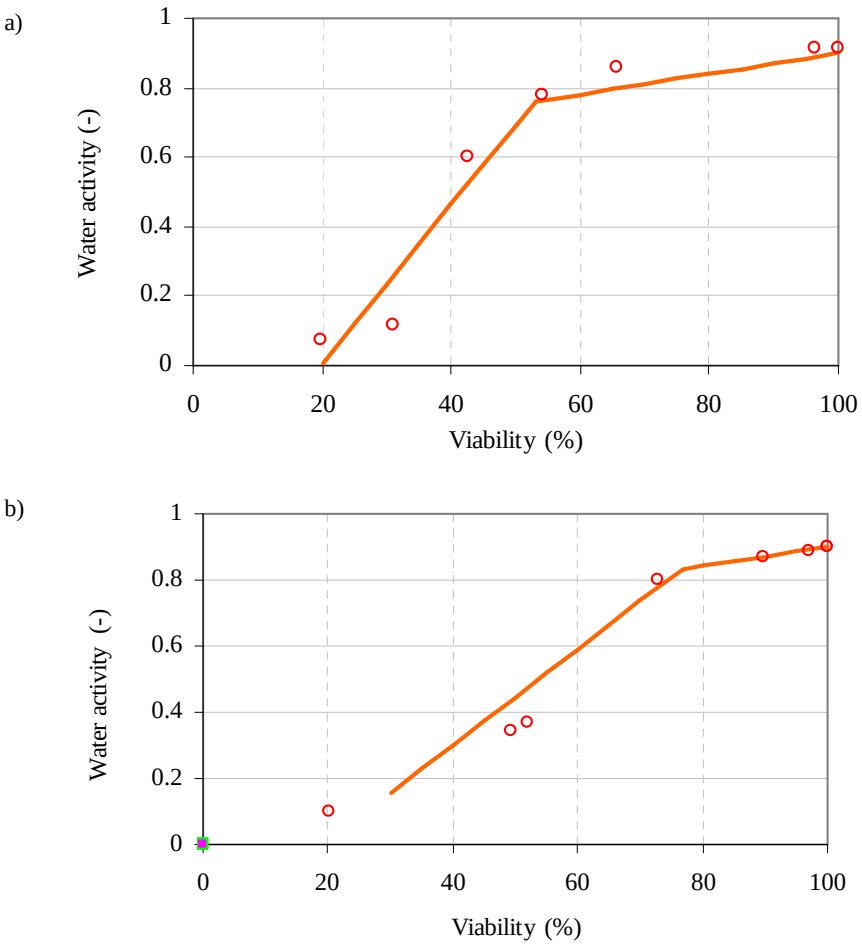

Fig. 1. Viability of yeast *Saccharomyces cerevisiae* freeze-dried without addition of lactose (a) and with lactose (b) as a function of water activity

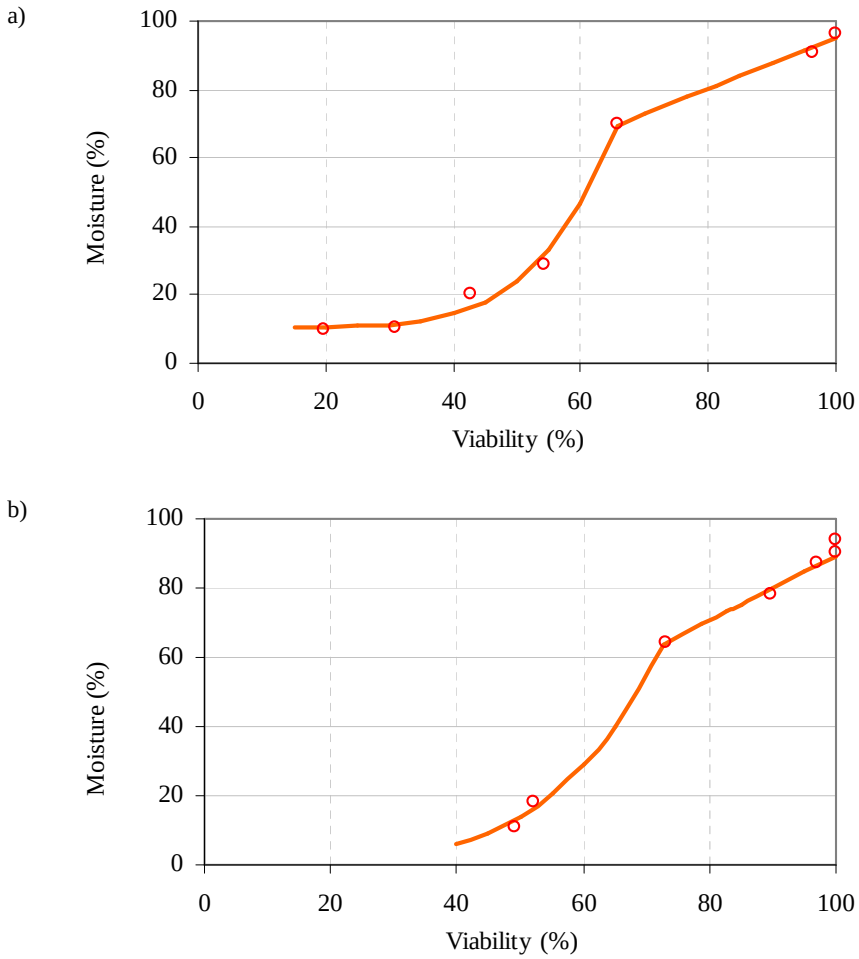

Fig. 2. Viability of yeast *Saccharomyces cerevisiae* freeze-dried without addition of lactose (a) and with lactose (b) as a function of water content

In all the samples under study the existence of a critical value of the water activity was found (0.762 in the samples without lactose and 0.767 in the samples with lactose), upon reaching which the survival rate of yeast cells was dynamically reduced, however, the lethal effect noticed was much lower in the samples with lactose than in those without lactose. Besides, it was impossible to post-dry the samples that contained lactose to a water activity lower than 0.346, which ensured the maintenance of the cell activity at a level of 49.3%, whereas the final water activity in the samples without lactose amounted to 0.074, and the survival rate to 19%.

The nature of variations in the viability of cells in the function of the material water contents was congenial in samples with and without lactose, and it was found that the dehydration of alginate-yeast beads followed two-stage-wise. A change in the process course from rectilinear in the first drying period to the exponential in the second drying period followed at the moisture contents amounting to 70% for samples without lactose and 64% for samples with lactose. Lyophilizates obtained were characterized by similar final moisture contents but the viability of cells dried with an additive of lactose was higher than for those dried without an additive of sugar.

The determination of the cell dying dynamics during the lyophilization process of alginate-yeast globules allows presuming that in order to reach some limit values for the water activity and moisture contents there followed the dehydration process mainly of calcium alginate while a high survival rate of cells was maintained. A drop in the viability of yeast being dried, as noticed at the second stage, can bear testimony to the intensification of the removal of ice from more strongly condensed cell solutions.

The aforementioned test results bear evidence to the impact of the lactose presence on the physiological status of yeast cells subjected to the dehydration process by the freeze-drying method. The survival rate of cells immobilized within calcium alginate with an additive of lactose was 30% higher than in samples without lactose.

Microwave-vacuum drying

The survival rate of yeast immobilized in calcium alginate at the beginning stage of the microwave-vacuum drying was gradually deteriorated despite the fact that the water activity was maintained at a constant level of $a_w = 0.90$ (Fig. 3.). This phenomenon resulted probably from the seamless temperature distribution within the whole volume of the material being dried, and consequently – simultaneous dehydration of alginate and yeast cells. The water present in beads permanently showed a high activity but a part of already dehydrated cells were damaged. The lethal effect was intensified in the presence of lactose.

The variation dynamics of water activity for the drying process of biomaterial without lactose were higher than for samples with lactose which hindered the maintenance of a high survival rate of cells. The final water activity of samples dried with lactose was considerably higher, on the other hand, the physiological condition upon completion of the drying process was worse than for samples without additional sugar.

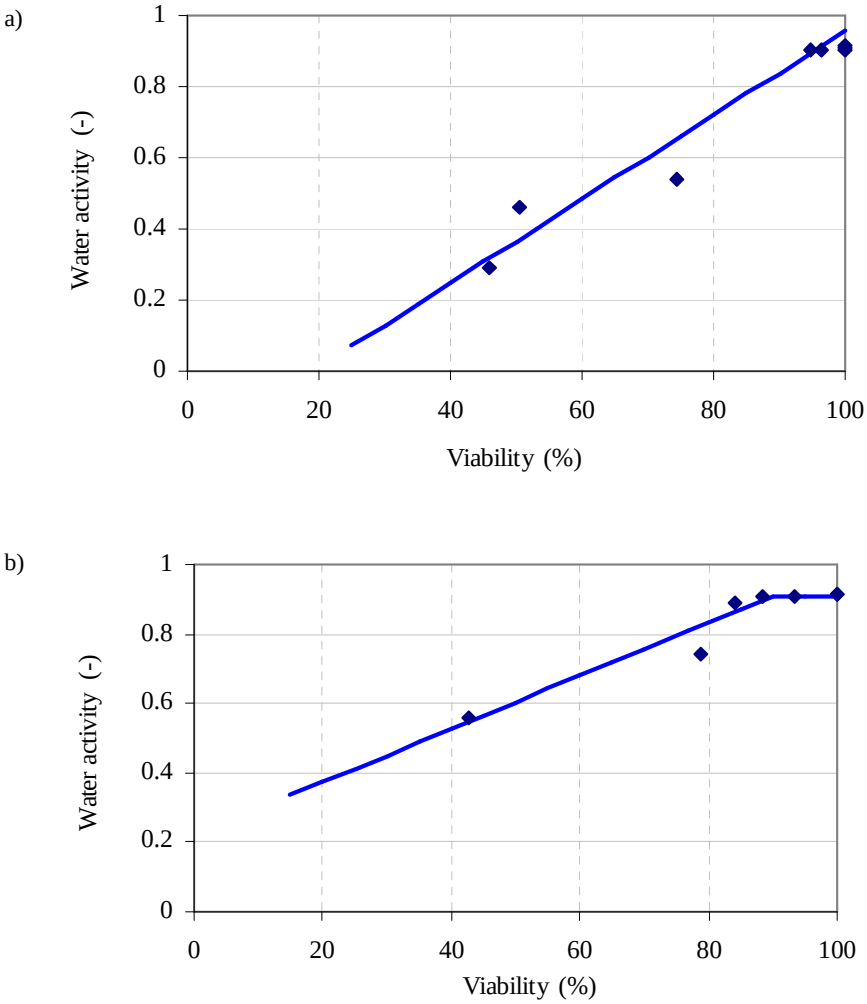

Fig. 3. Viability of yeast *Saccharomyces cerevisiae* during microwave-vacuum drying without addition of lactose (a) and with lactose (b) as a function of water activity

A dynamic drop in the moisture contents of the material being dried, both containing lactose and without lactose, followed until moisture content at a level of ca. 20% was reached (Fig. 4.). A minor change in the moisture contents of beads below that level produced a considerable lowering of the survival rate of cells.

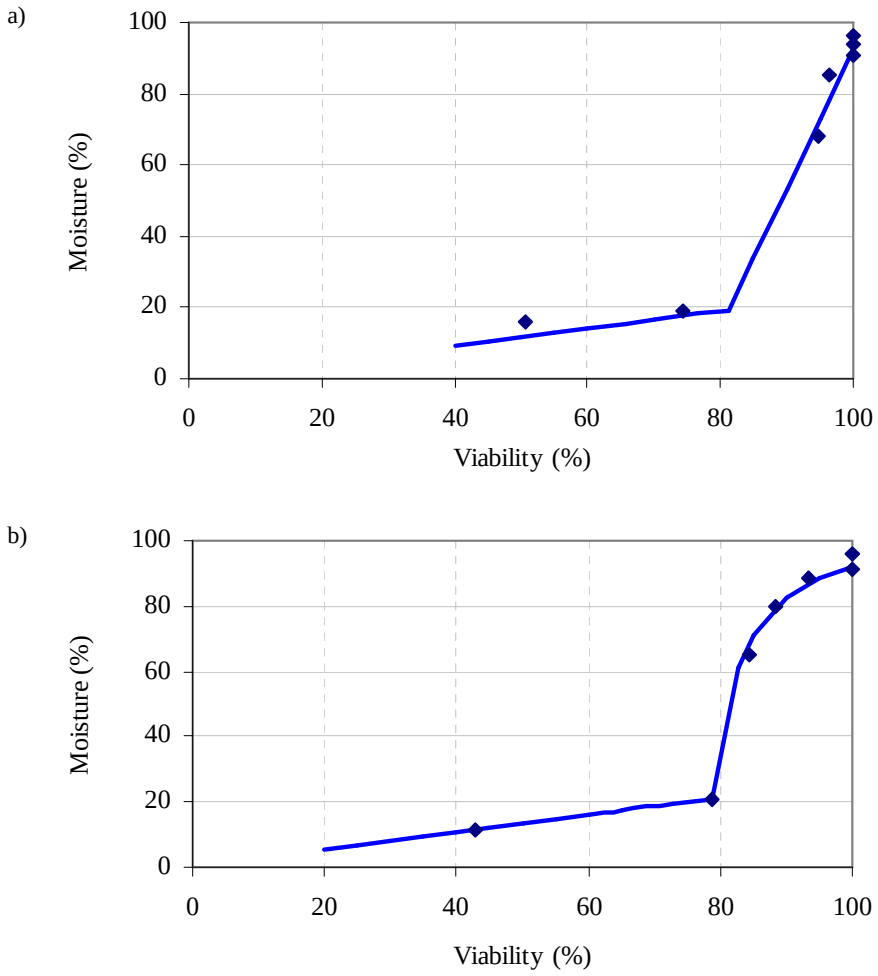

Fig. 4. Viability of yeast *Saccharomyces cerevisiae* during microwave-vacuum drying without addition of lactose (a) and with lactose (b) as a function of water content

DISCUSSION

The application of lactose as an osmoprotective compound in sublimation drying of yeast *Saccharomyces cerevisiae* resulted in an improvement of its survival rate. The precise mechanism of the participation of sugars in the maintenance of the integrity of biomaterials being dehydrated has not been fully explained, however, two hypotheses were set. According to the first one, sugars are

combined during the rehydration process with proteins and, by replacing water particles, they maintain the protein structure, not allowing for the denaturation process to follow (Saleki-Gerhardt *et al*. 1995). Basing on that theory, a conclusion can be drawn that the majority of mono-, di- and trisaccharides can be used for the protection of sensitive materials against the negative effects of the drying process. However, it has been demonstrated in practice that the effect of the biomaterials stability improvement (enzymes, liposomes, medicines and microorganisms) dried with various carbohydrates is differentiated. An alternative point of view, related to the positive impact of sugars on labile biological and biochemical systems (hypothesis number two), is based on the sugars tendency to get transformed into an amorphous vitreous state. The stable form of glass in itself stabilizes to a sufficient degree the biomaterial being dried. This phenomenon is based on the potential for the appearance of sugars in the form of sticky glass or, alternatively, in the form of a more fluid rubber – an oversaturated solution having a viscoelastic consistency (rubber state). A change from vitreous to fluid state follows at a temperature specific for each material and upon reducing that value, the migration of water steam particles from small particles to large particles is stopped, and the remaining intercrystalline solution achieves a maximum concentration of soluble substances and shows the features of an amorphous material. A further temperature reduction leads to over-cooling of the condensed liquid and a rapid growth in its viscosity. A structure similar to glass is formed between ice crystals (vitrification) (Slade *et al*. 1995). The testing performed seems to confirm the protective functions of lactose during the sublimation drying of yeast *Saccharomyces cerevisiae*.

On the other hand, an additive of lactose caused a reduction in the survival rate of cells of yeast *Saccharomyces cerevisiae* dried using a microwave-vacuum method. A probable cause was the fact that in systems in which sugars and proteins appear (majority of biological systems), a chemical reaction between those substances follows spontaneously (Maillard's reaction), this being a reason for the loss of a part of proteins (mainly L-lysines, glycins and albumin) from the total pool of dehydrated material (Buera *et al*. 1995). The lactose presence produced a rise in the intensity of the Maillard's reaction occurrence, also of the hydrolysis and the burning of sugars.

CONCLUSIONS

1. An addition of lactose causes improvement of viability of freeze-dried yeast *Saccharomyces cerevisiae* immobilized in Ca-alginate, however, the final water activity is relatively high, which can have a negative influence on its storage stability.

2. Viability of immobilized yeast dried using microwave-vacuum method in the presence of lactose was lower than that of yeast dried without the addition of potential protectant.

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ŻYWOTNOŚĆ DROŻDŻY *SACCHAROMYCES CEREVISIAE* ODWADNIANYCH W OBECNOŚCI LAKTOZY

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S t r e s z c z e n i e. Badano wpływ laktozy na żywotność drożdży *Saccharomyces cerevisiae* suszonych sublimacyjnie i mikrofalowo-próżniowo. Laktozę dodawano do zawiesiny drożdży, mieszaninę unieruchamiano w matrycy alginianu wapnia w postaci kulek, a następnie poddawano suszeniu. Stwierdzono, że dodatek cukru poprawia żywotności komórek suszonych w stanie zamrożenia, natomiast w przypadku suszenia mikrofalowo-próżniowego powoduje pogorszenie przeżywalności populacji.

S ł o w a k l u c z o w e: suszenie sublimacyjne, suszenie mikrofalowo-próżniowe, laktoza, drożdże