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EFFECT OF YEAST BIOMASS ENRICHMENT IN MAGNESIUM ON DRYING KINETICS AND SURVIVAL OF CELLS

Dorota Nowak[1], Tadeusz Kasiak[1], Piotr P. Lewicki[1], Wanda Duszkiewicz-Reinhard[2]

[1]Department of Food Engineering and Process Management, [2]Division of Biotechnology and Food Microbiology; Warsaw Agricultural University (SGGW), Warsaw

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Brewers yeast *Saccharomyces cerevisiae* were cultivated on defined growth medium supplemented with magnesium ions. Harvested cells were dried by convection under variable conditions and under vacuum. An increased content of magnesium in yeast cells affected the convective drying. The cells enriched in magnesium dried faster and their survival was increased in comparison to the control cells.

INTRODUCTION

Deficiency of magnesium ions, one of the most important microelements, has been recorded in about 50% of people. Research carried out on a laboratory scale has shown that yeast cells can be a good source of magnesium for human diet, because the formed bioplexes are well assimilated [Błażejak *et al.*, 2002b].

Saccharomyces yeast are considered as safe for human health, they are easy for cultivation, give high biomass yield and are not very demanding as far as the growth medium composition is concerned. Moreover, due to characteristic structure of the cell wall the yeast exhibit high ability to bind divalent ions [Lipke & Ovalle, 1998].

Yeast cells enriched in magnesium can be used as a biocatalist or as a source of nutrients. Hence, depending on the use, the cells should be either alive or dead. However, regardless of the way the yeast are used, the most convenient is the dry form of preparation. In comparison to pressed yeast, the dry preparation has much longer shelf-life, is enzymatically stable, and does not change its individual characteristics, which is very important for highly selective strains [Morakile *et al.*, 2002]. Reduced mass and volume, ease of dosing, packing and transportation are also the advantages of dry yeast preparations.

Dry yeast preparations used as biocatalysts must fulfil requirements for active dry yeast (ADY), *i.e.* the survival should be from 2.2×10^{10} to 2.5×10^{10} of alive cells in one gram of dry matter. The activity of a dry preparation should not decrease by more than 0.3% during one month of storage [Bayrock & Ingledew, 1997].

High activity of dry yeast preparation depends on the following variables: (1) pre-propagation and propagation conditions; (2) drying mode and parameters, and (3) conditions of reconstitution and restoration of activity.

Temperature of hot air is one of the variables affecting survival of yeast cells during drying. Bayrock and Ingledew [1997] reported that the survival of *Saccharomyces cerevisiae* cells during fluidised bed drying did not follow the kinetics of thermal death.

It is expected that the enrichment of yeast cell in magnesium can affect the structure of both, the cell wall and biopolymers, and result in different drying kinetics and survival in comparison to the cells propagated under ordinary conditions. Hence, the aim of this work was to assay the effect of yeast cell enrichment in magnesium on drying kinetics and survival of the cells in relation to drying variables.

MATERIAL AND METHODS

Production of biomass. Propagation of brewers yeast *Saccharomyces cerevisiae* No.1 obtained from the collection of pure cultures of the Division of Food Biotechnology and Microbiology of Warsaw Agricultural University (SGGW) was carried out in a bioreactor BIOFLO 3000 (New Brunswick Sci.). The growth medium YPD in the quantity of 5 L was supplemented with 1.25 g Mg^{2+}/L using $MgCl_2\cdot6H_2O$ as a source of magnesium. The amount and source of magnesium ions were recommended by Błażejak *et al.* [2002b]. Magnesium was added at the beginning of the propagation process – variant Mg1, or was added continuously during the first two hours of the process – variant Mg2. The Mg2 process was designed in order to adapt yeast

Author's address for correspondence: Dorota Nowak, Department of Food Engineering and Process Management, Warsaw Agricultural University, ul. Nowoursynowska 159c, 02-776 Warsaw, Poland; tel.: (48 22) 59 375 62; fax: (48 22) 59 375 76; e-mail: Nowak@alpha.sggw.waw.pl

cells to an increased magnesium concentration in the growth medium. Control propagation of yeast was carried out without the supplementation of the growth medium with magnesium (Contr). Inoculum was prepared in the YPD medium by inoculation with cells stored at 4°C on the slants. Propagation was carried out at 28°C for 22–24 h, until a constant optical density was reached, using a shaker at 200 rpm.

Bioreactor vessel filled with growth medium and equipped with oxygen and pH electrodes and a thermometer was sterilized at 121°C for 25 min. After cooling and polarisation of the oxygen electrode, the vessel was coupled to a controlling and programming unit. Calibration of oxygen electrode was done on a medium cooled down to a working temperature. Then the inoculum was added at 10% v/v.

The vessel was equipped with baffles and turbine agitator. Propagation of yeast cells was carried out at 400 rpm. The medium was aerated by sparger using filtered ambient air at 100 L/h. Under these conditions, a high level of oxygen concentration in the growth medium was assured.

Propagation was conducted for 24 h. Yeast cells were harvested by 10-min centrifugation at 4000 g. The concentration of the biomass harvested was *ca.* 23% d.m.

Drying of biomass. The yeast biomass was extruded as short cylinders with a diameter close to 2 mm. Four different modes of drying were applied; three of them were convective processes and the fourth one was based on drying under vacuum (P), at parameters of the triple point of water. Convective drying was carried out under the following conditions: temperature of hot air controlled in such a way that the temperature of biomass did not exceed 35°C (K35); biomass extruded with the addition of 10% w/w of rye flour and dried as above (M+K35), and biomass dried at constant hot air temperature of 70°C (K70).

Hot air was flowing along the material undergoing drying with the velocity of 1.6 m/s. A decrease in the mass of the material was recorded continuously with the use of POMIAR software Temperature of both, the material undergoing drying and hot air was scanned continuously with the TUXLAB equipment and recorded at a computer.

Vacuum drying was carried out in a freeze-dryer Alpha 1–4 (Christ) at the pressure of 630 Pa and shelf temperature of 10°C. Temperature of the material undergoing drying was scanned continuously with MPI-Lab equipment.

Dried biomass was stored in hermetic containers at room temperature for one month.

Analytical methods. The concentration of magnesium in yeast biomass washed twice with deionizated water was measured with atomic absorption spectroscopy (AAS). Dry matter content of freshly harvested and dried biomass was assayed by the two-step drying method. Initial drying was carried out at 60°C for 2 h and then the material was dried at 105°C until the constant mass was reached.

The number of alive cells was measured by the enumeration of colony forming units (cfu) related to 1 g d.m. of biomass. The known mass of the material was diluted with sterile water and then plated on the YPD medium containing 2% of agar. Incubation was carried out at 28°C for 72 h. The number of live cells was measured in freshly harvested biomass and in dry preparations after drying and after 1 month of storage.

RESULTS AND DISCUSSION

Preliminary propagation conducted for 72 h showed that the logarithmic phase of growth ends after 24 h. From the drying point of view, the yeast cells should be harvested during the stationary phase of growth [Gervais *et al.*, 1992], since they are less susceptible to stress than those actively multiplying. However, the results published by Błażejak *et al.* [2002a,b], who used the same strain of *Saccharomyces cerevisiae* as that investigated in this work, demonstrated that cells grown under static or dynamic conditions release magnesium ions to the growth medium during the stationary phase. Research done by Walker and Maynard [1997] and reporting on the kinetics of accumulation of magnesium by the *Saccharomyces cerevisiae* cells showed that at the end of the logarithmic phase of growth the cells begin to release the ions to the surrounding medium. Other results show that an increased content of magnesium increases the resistance of *Saccharomyces cerevisiae* cells to thermal and ethanol concentration stress. [Birch & Walker, 2000], which can persist as long as 24 h. Taking all the published results into account it was decided to interrupt the propagation after 24 h and harvest the cells which are at the end of the logarithmic phase of growth. It was expected that the cells would still contain an increased amount of magnesium ions, which in turn will result in better survival during drying.

Biomass obtained under control conditions contained 1.33 mg·(g d.m.)$^{-1}$ of magnesium. In the variants Mg1 and Mg2, magnesium content was 2.86 and 2.75 mg·(g d.m.)$^{-1}$, respectively. Hence, the biomass harvested from the medium magnesium enriched contained more than twice as much of the magnesium ions as that of the control cells.

Convective drying of biomass at 70°C (variant K70) lasted from 50 to 80 min, and final water content was dependent on the propagation conditions. Cells with an increased magnesium concentration dried to the final water content of 0.05 g·(g d.m.)$^{-1}$, while the control material dried to water content equal to 0.09 g·(g d.m.)$^{-1}$. In the variant K35, in order to keep material temperature below 35°C, the hot air temperature at the beginning of the process was 55°C and lowered gradually to 37°C. The process was long and the time to reach water content close to 0.15 g·(g d.m.)$^{-1}$ was from 270 to 350 min. When biomass was mixed with rye flour (variant M+K35), the drying time was shortened by about 20%, and the final water content was close to 0.10 g·(g d.m.)$^{-1}$.

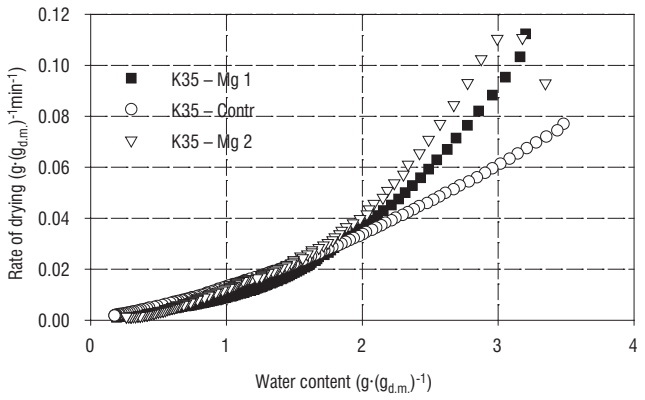
Drying under vacuum took about 5 h, and the final water content was close to 0.04 g·(g d.m.)$^{-1}$. There was no possibility to measure precisely the temperature of the drying material; hence the drying was terminated after the prescribed time. Under these conditions no effect of magnesium on the drying kinetics was found.

Propagation method and drying parameters affected the drying kinetics of the yeast biomass (Figure 1). The rate of drying at the same water content differs by as much as 3-fold.

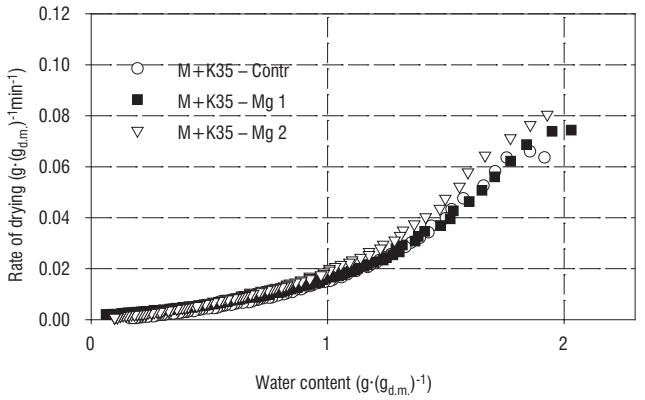
Regardless of the propagation method and drying conditions convective drying of yeast biomass proceeded in the period of falling rate of drying. The rate of drying was dependent on the content of magnesium ions, especially at the beginning of the drying process. The most pronounced differences occurred in the variant K35 (Figure 1a) and were statistically significant until 50% of water was evaporated from the material. At water concentrations lower than 65% there was no difference in the rate of drying of control cells and cells enriched in magnesium.

Rye flour added to the yeast biomass as the protecting material reduced differences in drying kinetics between control cells and cells enriched in magnesium ions (Figure 1b). The effect observed can be due to much lower initial water content of the material undergoing drying. The initial water content in the variant K35 was 3.2–3.5 g·(g d.m.)$^{-1}$, while in the variant M+K35 it was close to 2.0 g·(g d.m.)$^{-1}$. At that water content there were no significant differences in drying rates of materials dried in the variant K35. Drying rate at 70°C was also dependent on the presence of magnesium ions in the yeast cells. Cells enriched with magnesium dried much faster than the control ones (Figure 1c). The differences persisted until *ca.* 75% of water were evaporated from the material.

a)



b)



c)

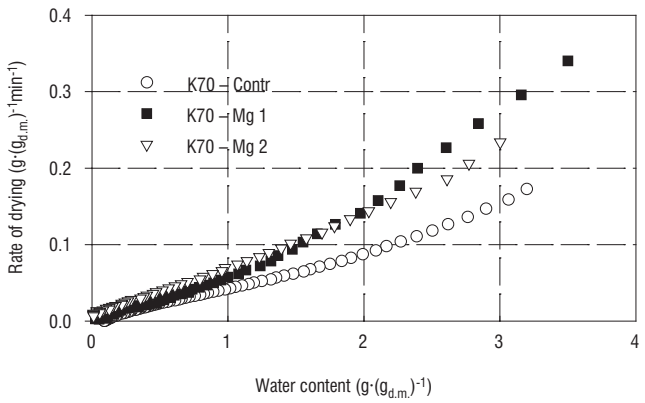


FIGURE 1. Relationship between the rate of drying and water content in yeast biomass under different drying conditions: a) variant K35, b) variant M+K35, c) variant K70.

The drying rate at water content of 3 g·(g d.m.)$^{-1}$ was from 0.15 to 0.30 g·(g d.m.·min)$^{-1}$ at 70°C and from 0.06 to 0.11 g·(g d.m.·min)$^{-1}$ at 35°C, the lower values being characteristic for the control yeast biomass. At water content of 2 g·(g d.m.)$^{-1}$ the drying rate was from 0.06 to 0.08 g·(g d.m.·min)$^{-1}$ in the variant M+K35, and from 0.035 to 0.04 g·(g d.m.·min)$^{-1}$ at 35°C. Hence, the convective drying variants differed substantially in the rate of drying.

The results show clearly that the increased magnesium content in yeast cells enhances convective drying, especially at the beginning of the process. The influence of magnesium ions on drying kinetics is very pronounced at high water contents and decreases with decreasing wetness of the yeast biomass. The effect can result from the influence of magnesium ions on the permeability of the cell membrane to water. Magnesium ions are absorbed on the cell wall and thereafter are transported to the cell. A difference in magnesium ions concentration across the cell wall is created, and the electrostatic interactions may affect cell membrane permeability. Moreover, the electrochemical potential formed on the cell wall can promote water movement [Walker & Maynard, 1997].

Biomass harvested after 24 h of propagation contained 2×10^{11} cfu in 1 g d.m. of control material. In variants Mg1 and Mg2 one gram of dry matter contained 2.9×10^{11} and 3.0×10^{11} cfu, respectively. It is evident that the supplementation of the growth medium with magnesium resulted in much higher yield of biomass.

Drying affected the survival of yeast cells. The highest survival was observed for the cells dried under vacuum (Figure 2). The number of cells decreased by one logarithmic cycle in the control material (it corresponds to 90%). In the variant Mg1 the decrease was by 30%, and in the Mg2 it amounted to *ca*. 60%.

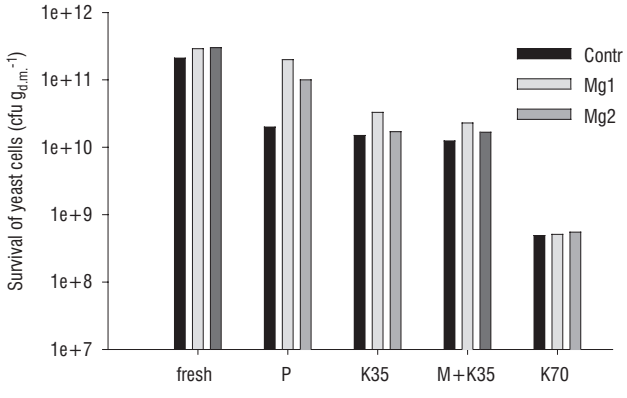


FIGURE 2. Influence of drying mode on the survival of yeast cells.

Survival of the control cells did not differ statistically for drying variants P, K35 and M+K35. However, in all these variants the survival of cells enriched with magnesium was higher than that in control material. It is evident that magnesium ions increased the resistance of yeast cells to the stress created by the drying process. Drying at 70°C reduced the number of surviving cells by *ca.* three logarithmic cycles, and the protective effect of magnesium was no longer evident. Dry preparations obtained on variants P, K35 and M+K35 fulfilled the requirement for the ADY [Bayrock & Ingledew, 1997]. The increased survival of cells enriched in magnesium ions can be due to the reduced damage of the cell wall caused by thermal and water concentration stress. Brich and Walker [2000] observed, by scanning electron microscopy, the protective effect of magnesium on the cell wall upon thermal stresses.

Storage of dry preparations caused reduction in surviving cells by one logarithmic cycle in the variants K35 and K75. In the variant P and M+K35, the reduction was close to 10%. The final water content of the dry preparations was different. For variants P and M+K35 it was from 0.04 to 0.10 g·(g d.m.)$^{-1}$, while in dry material from variant K35 it was about 0.15 g·(g d.m.)$^{-1}$. Thus, the stability of dry preparations may be a function of water content and should be a subject of further research.

CONCLUSIONS

The increased content of magnesium ions in the biomass of brewers yeast intensifies convective drying, especially at the beginning of the process.

Under drying conditions assuring high survival of yeast cells the presence of magnesium ions increases the resistance of the cells to drying stresses. Hence, the survival of cells enriched in magnesium can be by 50% higher than that of the control material.

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WPŁYW WZBOGACENIA BIOMASY DROŻDŻY W MAGNEZ NA KINETYKĘ SUSZENIA I PRZEŻYWALNOŚĆ KOMÓREK

Dorota Nowak[1], Tadeusz Kasiak[1], Piotr P. Lewicki[1], Wanda Duszkiewicz-Reinhard[2]

[1]Katedra Inżynierii Żywności i Organizacji Produkcji, [2]Zakład Biotechnologii i Mikrobiologii Żywności Katedry Biotechnologii, Mikrobiologii i Oceny Żywności, Wydział Technologii Żywności Szkoły Głównej Gospodarstwa Wiejskiego, Warszawa

W pracy poddano suszeniu drożdże piwowarskie *Saccharomyces cerevisiae* wyprodukowane w biofermentorze na zdefiniowanym podłożu kontrolnym i wzbogaconym w magnez. Zastosowano 3 warianty suszenia konwekcyjnego oraz suszenie próżniowe. Stwierdzono, że zwiększona zawartość magnezu w komórkach wpływa na zwiększenie szybkości suszenia (rys. 1). Zwiększony poziom magnezu powodował również lepszą przeżywalność komórek w trakcie zastosowanych suszeń (rys. 2).