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AN ATTEMPT TO LOCALISE MAGNESIUM IN THE CELLS OF BAKER'S YEASTS *SACCHAROMYCES CEREVISIAE* NO. *102* SUPPLEMENTED WITH THIS ELEMENT

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ABSTRACT

The study aimed at determining which part of magnesium permanently bound with baker's yeasts *S. cerevisiae* No. *102* during batch culture in control (YPD) and experimental medium (YPD with the addition of magnesium) remains in the cell wall and which undergoes intracellular bio-accumulation. The experimental media were supplemented with the amount of $MgCl_2*6H_2O$ providing 1.25 g/dm^3 content of Mg^{2+} ions. The cultures were run for 24 h at 28°C in a reciprocating shaker which provided aerobic conditions of the process. The application of enzymatic protoplastisation and mechanical disintegration of the yeast cells (at a temperature of liquid nitrogen) enabled determination of the contents of magnesium and total protein in both cell walls and protoplasts. The yeasts from a 24 h batch culture in the control YPD medium (without magnesium) demonstrated diversified contents of magnesium in the cell walls (1.84 mg Mg^{2+}/g d.m. of yeast) and protoplasts (0.36 mg Mg^{2+}/g d.m. of yeast). Similarly, in the cell biomass from the culture grown in the experimental media (enriched with magnesium) most of the magnesium ions (5.56 mg Mg^{2+}/g d.m. of yeast) were located in the cell walls, while as little as 1.08 mg Mg^{2+}/g d.m. were identified in the protoplasts, which constituted 84% and 16%, respectively, of the total content of magnesium permanently bound with the yeasts. The 1.25 g/dm^3 addition of Mg^{2+} ions to the experimental media evoked a significant (35%) increase in the total protein content in the yeast cells compared to the control medium culture.

Key words: bioplexes, metalloproteins, magnesium, bio-elements, *Saccharomyces cerevisiae*.

INTRODUCTION

Proper balancing of the diet components is of crucial importance to the human health. Macro- and micro-elements, including magnesium, play important roles in the human organism functioning on the cellular level. A contemporary diet often contains highly-processed products, which in turn results in too little amounts of magnesium provided to the organism [20]. Supplementation of a human diet with this deficient element seems therefore necessary. In recent years attention has been paid to bio-accumulating capability of yeasts towards microelements. The specific structure of the cell wall makes the fungi possible to bind on their surface ions of metals occurring in the environment. Due to some cellular mechanisms the cations adsorbed can be transported to the inner part of the yeast cells and occur therein in the form of bioplexes, namely durable bonds of metals with proteins or amino acids [9, 21, 23, 25].

The literature data [16, 24] point to a substantially higher availability of metal ions from protein complexes than from organic or non-organic salts of these elements by human and animal organisms. It is explained by the fact that the transporting routes of magnesium occurring in the form of a bioplex are specific to protein or amino acids, but not to a cation. The bioplex remains soluble and stable within a wide pH range which occurs in the gastrointestinal tract. Unlike magnesium in the form of Mg^{2+} ions, it easily penetrates the intestinal wall and then plasmatic membranes of the cells.

Two types of proteins Alr1p and Alr2p take part in active transportation of magnesium to the inner part of the yeast cells [15]. They belong to the family of membrane integral proteins (MIT), i.e. proteins constituting a part of cytoplasmatic membrane and enabling transportation of metal ions on the basis of enhanced diffusion [4]. The Alr transporters are efficient, they may transport cations of different elements (zinc, cobalt, manganese), however they demonstrate a specific affinity to the magnesium. Mg^{2+} ions binding with those transportation proteins can be good source of these elements for human and animal organisms by their a high availability.

The objective of this study was to localise magnesium in the cells of baker's yeasts *Saccharomyces cerevisiae* No. *102* supplemented with this element during a batch culture in the experimental medium with the addition of Mg^{2+} ions in the form of chloride salt. Determinations were performed to identify which part of the magnesium bound with the yeast cells was present in the cell wall and which underwent intracellular bio-accumulation. The following experimental procedures were run to accomplish the objective of the paper:

- culture of baker's yeasts *S. cerevisiae* No. *102* in a control (without additional source of magnesium) and experimental medium (with the addition of magnesium),
- enzymatic protoplastisation of the yeast cells from the control and experimental media,
- mechanical disintegration of the yeast cells from the control and experimental media with the use of liquid nitrogen,
- determinations of the contents of Mg^{2+} ions and total protein.

MATERIALS AND METHODS

Biological material

The study was performed on the strain of baker's yeasts *Saccharomyces cerevisiae No. 102* originating from the pure culture collection of the Department of Food Biotechnology and Microbiology, SGGW, Warsaw, Poland. The yeasts were stored in YPD slants at a temperature of +4 C.

Microbiological media

- the YPD medium with 2% agar [19, 22] – used for storing the yeasts in slants and determining the yeast number with a plate method,
- the control medium – liquid YPD medium – used for preparation of *inoculum* and batch culture of baker's yeasts,
- the experimental medium – liquid YPD medium supplemented with 1.25 g/dm^3 of Mg^{2+} ions – used for enriching the baker's yeast cells with magnesium during the batch culture.

Lytic enzymes

The following lytic enzyme was used for the protoplastisation of the yeast cells: Lyticase, Sigma, catalogue No. L 2524. This enzyme, obtained from the extract of *Arthrobacter luteus* bacteria, is able to hydrolyse β-(1→3)-glycoside bonds occurring in polysaccharides which form the structure of the yeast cell walls. The preparation used was in the form of lyophilised powder with the activity of 1420 A.U./mg.

Analytical assays

Determination of the yeast cell number
The number of the yeast cells was identified with the plate method in the YPD medium with 2% agar by performing batch inocula from three consecutive decimal dilutions [5]. Counting with the plate method was applied for determination of the degree of yeast protoplastisation with the lytic enzyme.

Determination of optical density (OD)
The optical density (OD) determination was performed to introduce similar numbers of the yeasts cell from the *inoculum* to the control and experimental media. The measurements of OD were carried out in a spectrophotometer (Spectronic 20 Genesys, USA) at the wave length of 600 nm [18].

Determination of the yield of the yeast cell biomass
The determination was performed by weighing the cell biomass from respective cultures, following centrifugation at 3500 rev./min for 10 min (Centrifuge MPW-365, Poland). The supernatant was decanted, and the centrifuged biomass was dried at 105°C to the constant mass.

Enzymatic protoplastisation of the cells of *S. cerevisiae* No. 102 baker's yeasts
Obtaining the protoplasts of the yeasts cell from 24-h cultures in the control (YPD) and experimental media (YPD supplemented with magnesium) ensured determination of these Mg^{2+} ions which broke the barrier of the cell wall and entered the inner part of the cell. In order to obtain cells devoid of the cell wall, the use was made of our own modification of the enzymatic procedure for protoplastisation by the Promega company, elaborated for DNA isolation [materials of the Promega company 2000]. The procedures included the preparation of reagents and performance of protoplastisation.

Reagents

- lyticase with the concentration of 70 mg/cm^3 (745.5 enzyme units were used for protoplastisation),
- EDTA with the concentration of 50 mM and pH 8.0 in 1 M of sorbitol (a buffer for suspension of the sediment, playing the function of an osmotic stabiliser).

Protoplastisation

- centrifugation (in an Eppendorf test-tube) of 1 cm^3 of 24 h yeast culture from either control or experimental medium and flushing twice with deionised water (8000 rev./min for 2 min),
- removal of supernatant and suspension of the centrifuged cell biomass in 293 μL and 7.5 μL of lyticase solution,
- incubation in a thermomixer (Eppendorf Thermomixer comfort, Germany) at 400 rpm and temperature of 37°C for 20, 40 or 60 min (in this time, the yeast cell walls are subject to protoplastisation),
- addition of 700 μL of 1 M sorbitol to the mixture,
- centrifugation of the sediment at 3000 rev./ min for 2 min,
- flushing twice with 1 M sorbitol and centrifugation of the sediment containing the mixture of protoplasts and not destroyed yeast cell.

Determination of the enzymatic protoplastisation degree of the cells of baker's yeasts *S. cerevisiae* No. *102*
The protoplastisation degree of the yeast cells was determined with the plate method. In a respective series of assays, two samples were collected for determinations: specific and control. The control sample (the yeasts from a 24 h culture in the YPD medium or the YPD medium supplemented with 1.25 g/dm^3 of Mg^{2+} ions) was not supplemented with the lytic enzyme. It was identified for the yeast cell number only. The specific sample collected simultaneously was supplemented with the solution of the lytic enzyme and in specified time intervals (after 20, 40, and 60 min of protoplastisation) inoculated into the YPD medium. It was assumed that the yeast cells which underwent protoplastisation upon the lytic enzyme activity would not grow in the standard YPD

medium used for the cell count in the plate method. The degree of the protoplastisation of the *S. cerevisiae* No. *102* cell was calculated as follows:

$$P\ [\%] = [\ (N_i - N_f) / N_i\ ] \times 100\%$$

where:
P – per cent of yeast cells which underwent protoplastisation [%],
N_i – initial number of yeast cells in the control sample prior to protoplastisation [cfu/ cm^3],
N_f – final number of yeast cell in the specific sample after protoplastisation [cfu/ cm^3].

Determination of the magnesium content in the cell biomass of yeasts and in the mixture of protoplasts with the yeast cells with the method of atomic absorption spectrophotometry (AAS)

For determination of the magnesium content in the cell biomass of yeasts and the mixture of protoplasts with the yeast cells, obtained upon enzymatic protoplastisation with lyticase, the samples were collected from 24 h batch cultures of *S. cerevisiae* No. *102* in the control and experimental media. Centrifuged, dried, and weighed biomass of the yeast cells or mixture of protoplasts with cells were mineralised by burning in the mixture of nitric and perichloric acids (Bűchi Digestion Unit K-435, Germany). In so prepared samples, the content of magnesium was assayed with the atomic absorption spectroscopy (AAS) method (Schimadzu AA660 spectrophotometer, Japan) [3]. The absorption was measured at the wave length of 285.2 nm. Taking into account the dilution and the weight of the dried samples, the results obtained in ppm were expressed as mg Mg^{2+}/g d.m. of the yeast cell biomass or mg Mg^{2+}/g d.m. of the protoplast-yeast cell mixture.

The following assumptions were made to determine the magnesium content in the cell wall [mg Mg^{2+}/g d.m. of yeasts] and protoplasts [mg Mg^{2+}/g d.m. of yeasts]:

- the content of Mg^{2+} ions in dry matter of the yeasts was the sum of the magnesium ion contents in the dry matter of cell walls and protoplasts,
- the dry matter of the cell walls constituted 20% of the dry matter of the yeast cells [13],
- the product of the protoplastisation degree of the yeast cells - P [%] and percentage of the cell walls in the yeast dry matter [%] indicated this part of the cell dry matter which together with magnesium passed into the supernatant after centrifugation of lytic enzyme-protoplastisised samples of yeasts.

On this basis, the following relationships were used in the calculations:

1. $Mg_{cw} + Mg_p = Mg_c$
2. $[(1 - P) \times Mg_{cw} + Mg_p] / 1 - P \times 0.2 = Mg_{p+c}$

where:
Mg_{cw} – mg Mg^{2+} in the cell wall / g d.m. of yeasts,
Mg_p – mg Mg^{2+} in the protoplast / g d.m. of yeasts,
Mg_c – mg Mg^{2+} in the yeast cells/ g d.m. of yeasts,
Mg_{p+c} – mg Mg^{2+}/ g d.m. of the mixture of protoplasts and yeast cells after 60 min activity of the lytic enzyme,
P – protoplastisation degree of the yeast cells after 60 min activity of the lytic enzyme [%],
1 – P – the cells which did not undergo protoplastisation [%],
1 – P x 0.2 – the dry matter of yeasts contained in the mixture of protoplasts and not destroyed cells which were not susceptible to the activity of the lytic enzyme for 60 min [%].

Disintegration of the cell of baker's yeasts *S. cerevisiae* No. *102* with liquid nitrogen
The disintegration of the yeast cells with liquid nitrogen enabled determination of the total protein content in the cell wall as well as in the protoplasts of *S. cerevisiae* cells. A sample of the cell biomass was collected from a 24 h culture of the yeasts in the control or experimental medium, centrifuged, transferred into a ceramic mortar, supplemented with liquid nitrogen (-196°C), and ground for ca. 4 min. The ground cell biomass, being flushed with deionised water, was transferred to an extraction thimble and centrifuged. After removal of the supernatant, the centrifuged sediment contained the so-called "pellet" which in turn contained fragments of the yeast cell walls.

Determinations of total nitrogen in the cell biomass and cell walls of *S. cerevisiae* No. *102* yeasts
The determination was performed according to the Kjeldahl method [11]. The centrifuged, dried and weighed cell biomass or fragments of the yeast cell walls (after disintegration of the cells with liquid nitrogen) were mineralised by burning in the concentrated sulfuric acid. After mineralisation, the samples were diluted,

neutralised, and ammonia with water vapour were distilled off (Bűchi 316 Distillation unit, Germany). The content of nitrogen in the samples was calculated into total protein with the use of a universal multiplier 6.25. The results of the total protein content were expressed in g protein / 100 g d.m. of yeasts for the cell biomass, and in g protein in the cell wall / 100 g d.m. for the cell walls (upon consideration of the previous assumption that the cell wall constituted 20% of the yeast cell dry matter). The difference between the protein contents in the cells and cell walls was used to compute its content in the protoplasts calculated into 100 g d.m. of yeasts.

Statistical analysis

A part of the results was subjected to statistical analysis performed with a computer programme Statgraphics Plus ver. 4.1. The analyses covered the significance of the effect of Mg^{2+} ions addition (1.25 g/dm^3 of medium, in the form of chloride salt) on the magnesium content in the cell walls and protoplasts of the *S. cerevisiae* No. *102* cells under the conditions of a batch culture. Attention was also paid to the evaluation of the effect of the magnesium dose added to the control medium on the total synthesis of cellular proteins of the investigated strain of baker's yeasts. Comparison of mean values was performed with the Tukey test at a significance level of $\alpha = 0.05$. The results of the statistical analysis (the least significant differences between the compared means, the so-called LSD) were marked in Tables 4-5.

Course of the study and discussion of more important results

This paper continues the studies performed at the Department of Food Biotechnology and Microbiology, SGGW, Warsaw, Poland, on binding some bio-elements by the selected strains of yeasts [8]. An attempt was made to answer the question: which part of magnesium assimilated by the yeast cells from culture medium binds permanently with the cell wall and which part – with the protoplast? The results obtained enabled location of the magnesium, supplemented to the experimental media, in the yeast cells. So as to obtain a high yield of the yeast biomass and high degree of cell enrichment with Mg^{2+} ions, a batch culture was run in the YPD medium. The type of the medium used as well as aerobic conditions of the culture ensured optimal growth of the yeasts [12].

The *inoculum* was prepared by slant inoculation of the liquid YPD medium with the experimental material. The yeasts were multiplied for ca. 24 h at a temperature of 28°C in a reciprocating shaker, at an oscillation frequency of 200 cycles/min (E. Bűhler SM-30 Control, Germany). Under such conditions, the optimal density (OD) of the culture medium reached 1.910. So obtained *inoculum* constituted the initial material for inoculation of the control and experimental media in the selected series of the experiment.

For the batch culture of the yeasts, the experimental media were supplemented with a dose of $MgCl_2*6H_2O$ able to ensure the final content of Mg^{2+} reaching 1.25 g/dm^3 of the medium after introduction of 10% (v/v) *inoculum*. The studies performed in the previous years [2], indicated that the magnesium salt applied in the described concentration enabled obtaining a high biomass yield and high content of magnesium permanently bound with the yeast cells, compared to the culture in the control medium (YPD without the addition of Mg^{2+} ions).

The yeasts from a 24 h culture in the control and experimental media were subjected to protoplastisation with a lytic enzyme. The obtained mixture of protoplasts and not destroyed cells was flushed with deionised water and dried. The contents of magnesium in the cell biomass and protoplast-cell mixture were determined with the AAS method. The results obtained and the known degree of yeast protoplastisation with lyticase enabled determination of the Mg^{2+} content in the cell wall.

At the beginning of the experimental part of the study it was assumed that the yeast samples collected should be in the final stage of logarithmic growth. Usually, the most intensive binding of magnesium from the medium by the yeast cells occurs in the first hours of culturing and sustains up to the end of the logarithmic phase [25]. The course of the protoplastisation process depends on the number of cells subjected to the activity of the lytic enzyme. Their numbers from both the control and experimental media should therefore be alike. Otherwise, the difference in the cell protoplastisation degree would be due to not only different magnesium content in the yeasts from the control and experimental cultures but also to disimilar number of the cells in relation to the lyticase dose applied. Determination of an approximate time of termination of the logarithmic growth phase as well as verification whether this moment occurs in the same time in the control and experimental media was therefore necessary.

Thus, measurements of optical density (OD) changes were performed during a 72 h batch culture of baker's yeasts *S. cerevisiae* No. *102* in the control (YPD) and experimental (YPD + 1.25 g Mg^{2+}/dm^3) media. The course of optical density changes (Table 1) indicated that the termination of the intensive growth occurred approximately in the 24th hour of culturing. Then the OD value reached ca. 2.3 for the culture both in the control and experimental medium. Taking into account that with extended time of culture the changes in the OD values

of both media were negligible and that the OD values after 24 h of culture proved similar numbers of the yeast cells in the media analysed, the 24 h cultures were found the most appropriate for further analyses specified in the aims of the paper.

Table 1. Changes of the optical density (OD) in the control (YPD) and experimental (YPD enriched in magnesium ions) media during cultivation *S. cerevisiae* bakery strain

Type of medium	Cultivation time [h]							
	0	2	4	6	24	30	48	72
YPD (control)	0.696	0.917	1.135	1.681	2.332	2.367	2.392	2.395
YPD + 1.25 g Mg^{2+}/dm^3	0.696	0.916	1.276	1.596	2.267	2.480	2.333	2.341

Optimisation of the protoplastisation conditions of *S. cerevisiae* No. *102* yeasts
An essential part of the undertaken study was the process of yeast protoplastisation. This technique was applied to separate the cell walls and obtain protoplasts. This process enabled the determination of the magnesium content in the protoplasts and then calculation of the magnesium content in the cell wall.

In the studies where removal of the cell wall from the cells of microorganisms is necessary, enzymatic protoplastisation is usually applied. It allows obtaining a relatively high percentage of wall-free cells. At the same time this method is not too invasive and upon appropriate parameters most of protoplasts remain not destroyed.

Optimisation of the protoplastisation conditions of *S. cerevisiae* No. *102* yeasts from the control and experimental media aimed at obtaining an equal and at the same time the highest possible degree of the cell protoplastisation. Due to a high cost of the enzymatic preparation, decision was made not to manipulate with the enzyme doses but with the time of protoplastisation process only. Four series of protoplastisation were performed for the yeast cells from cultures in the control and experimental media and their results were presented in Tables 2 and 3. A higher susceptibility to the lyticase activity was reported for the yeast cells from the control medium (YPD), compared to these from the experimental one (YPD + 1.25 g Mg^{2+}/dm^3). The highest gain in the protoplast number of the yeasts from the control and experimental cultures was observed in the first 20 min of the lytic enzyme activity on the cells. A total of 40% and 26% of protoplasts were obtained from cultures in the control and experimental media, respectively (Table 3). In subsequent 20 min of the yeast cell incubation with lyticase, the degree of protoplastisation increased up to 65% for the cell from the control culture and up to 42% for these from the experimental culture. In the last 20 min of the enzyme activity, the protoplastisation degree increased negligibly (up to 69% for the YPD-medium yeasts and up to 48% for the magnesium-enriched medium yeasts). Unequivocal explanation of such a decrease in the enzyme activity is quite difficult. Probably, the decrease could be due to a partial inactivation of the enzyme as a result of releasing metal ions or other molecules from the cell walls being decomposed. It can be also assumed that a part of the yeast cells had more stable walls, thus in the first 40 min lyticase decomposed the walls of the cells more susceptible to its activity, and the remaining cells underwent lysis less easily.

Table 2. Changes number of cells of *S. cerevisiae* bakery strain from the control and experimental media during incubation with lyticase

Incubation time [min]	Number of the yeast cell [cfu/cm^3]				
	Series I	Series II	Series III	Series IV	Means values
The yeast cell from YPD (control medium)					
0	5.10×10^8	5.20×10^8	5.40×10^8	5.00×10^8	5.20×10^8
20	3.21×10^8	3.07×10^8	3.08×10^8	3.05×10^8	3.10×10^8
40	1.99×10^8	1.66×10^8	1.67×10^8	1.90×10^8	1.81×10^8
60	1.79×10^8	1.56×10^8	1.46×10^8	1.60×10^8	1.60×10^8
The yeast cell from YPD +1.25g mg^{2+}/dm^3					
0	4.60×10^8	4.70×10^8	4.90×10^8	4.20×10^8	4.60×10^8
20	3.22×10^8	3.62×10^8	3.48×10^8	3.28×10^8	3.40×10^8
40	2.44×10^8	2.82×10^8	2.74×10^8	2.65×10^8	2.66×10^8
60	2.12×10^8	2.68×10^8	2.30×10^8	2.45×10^8	2.39×10^8

Table 3. Changes of protolplastisation degree of cells of *S. cerevisiae* bakery strain from the control and experimental media during incubation with lyticase

Incubation time [min]	Protoplastisation degree of the yeast cell [%]				
	Series I	Series II	Series III	Series IV	Means values
The yeast cell from YPD (control medium)					
20	37	41	43	39	40
40	61	68	69	62	65
60	65	70	73	68	69
The yeast cell from YPD +1.25g mg^{2+}/dm^3					
20	30	23	29	22	26
40	47	40	44	37	42
60	54	43	53	42	48

In this study, the addition of Mg^{2+} ions to the culture medium in the amount of 1.25 g/dm^3 had a significant effect on the protoplastisation degree of the baker's yeast cells (Table 3). In that case, no equal degree of protoplastisation of the yeasts from both the control and experimental media was obtained at the assumed concentration of the lytic enzyme. In the further part of the experiment, decision was made to run the protoplastisation for 60 min as only in this time the highest percentage of the wall-free cells was obtained. These observations were confirmed in the reports on the beneficial activity of magnesium on the stability of the yeast cell walls [1, 7]. Taking into account the equal (10% v/v) inoculation of the *inoculum* to the control and experimental media, it can be observed that decomposition of the cell wall with the enzymatic method in a similar number of the cells (Table 2) took twice as much time in the magnesium-supplemented yeasts as in the yeast from the control medium (Table 3).

The content of magnesium in the yeast cell biomass and in the mixture of protoplasts and cells of *S. cerevisiae* No. *102*
This part of the study aimed at determining which part of the magnesium was permanently bound with the cell wall structures and which underwent intracellular bio-accumulation during the batch culture of the yeasts in the control and experimental media. The comparison of the magnesium contents in the cell biomass, protoplasts, and cell walls of baker's yeasts depending on the culture medium used was presented in Table 4.

Table 4. The comparision of the contents and percentage part of nmagnesium ions between wall cell and protoplast of *S. cerevisiae* bakery strain from the control and experimental media

Type of medium/series	Contens of magnesium [mg Mg^{2+}/g d.w.]					Contens of magnesium [%]	
	Yeast cell [mg/g d.w.] yeast]	Protoplasts and yeast cells [mg/g d.w. protoplasts and cells]	Protoplastisation degree after 60 min [%]	Yeast wall [mg/g d.w. yeast]	Protoplast [mg/g d.w. yeast]	Yeast wall	protoplast
YPD							
Series I	1.95	1.36	69	1.67	0.28	86	14
Series II	2.46	1.69	69	2.07	0.39	84	16
Series III	2.30	1.58	69	1.94	0.36	84	16
Series IV	2.10	1.37	69	1.68	0.42	80	20
Means values	2.20c*	1.50	69	1.84c	0.36a	84	16
YPD+1.25g Mg^{2+}/dm^3							
Series I	6.60	3.09	48	5.56	1.04	84	16
Series II	6.01	2.88	48	5.18	0.83	86	14
Series III	7.10	3.21	48	5.78	1.32	81	19
Series IV	6.84	3.17	48	5.71	1.13	83	17
Means values	6.64e	3.09	48	5.56d	1.08b	84	16
	*Means with the same le48tter did not differ significantly						

The content of magnesium in the yeasts grown in the control medium (6.64 mg Mg^{2+}/g d.m.) was threefold higher than its content in the cell biomass from the control medium culture (2.20 mg Mg^{2+}/g d.m.). In the yeast cells from the experimental medium, most of Mg^{2+} ions (5.56 mg Mg^{2+}/g d.m. of yeasts) were bound to the cell wall. Magnesium located in the protoplasts in the amount of 1.08 mg Mg^{2+}/g d.m. of yeasts constituted as little as 16% of the total magnesium content in the cells (Table 4).

In the yeasts obtained from the control medium (YPD), distribution of the cellular pool of magnesium was identical as that reported for the experimental medium (Table 4). The mean magnesium content in protoplasts reached 0.36 mg Mg^{2+}/g d.m. of yeasts, compared to 2.20 mg Mg^{2+}/g d.m. of magnesium reported in the whole cells. Magnesium located in the protoplasts constituted 16% and that bound to the cell wall – 84% of the total magnesium content in the cells.

Obtaining an equal distribution of magnesium (permanently bound with the yeasts) in the cell walls and protoplasts both from the control and experimental media suggests that the magnesium dose applied (1.25 g Mg^{2+}/dm^3) did not break the metabolic equilibrium of the investigated yeast strain. This result indicates the possibility of undertaking attempts to enrich the yeast cell biomass with higher doses of magnesium being applied in the culture media.

Despite the fact that the yeasts from the experimental medium bound as much as 6.64 mg Mg^{2+}/g d.m. (trice as much as these from the control YPD medium), as little as 1.08 mg Mg^{2+}/g d.m. underwent intracellular bio-accumulation during the 24 h batch culture. Transportation of magnesium to the inner part of the cell proceeds by enhanced diffusion and requires a high energy expenditure connected with biosynthesis of protein [10, 15]. Perhaps extending the culture time in the experimental medium would allow transporting a higher number of Mg^{2+}ions, bound with the cell wall surface on the basis of chemisorption, to the inner parts of the cells.

The effect of *S. cerevisiae* No. *102* yeasts supplementation with magnesium on the total protein content in protoplasts and cell walls

The presence of Mg^{2+} ions in the environment may affect expression of genes responsible for the production of transporting proteins of the cytoplasmatic membrane [15, 17]. On the basis of these reports, a comparative analysis was performed with respect to the total protein content (in protoplasts and cell walls) in the yeasts from a 24 h batch culture in the control and experimental media. The results of the total protein content in the yeast cell biomass, protoplasts, and cell wall were compiled in Table 5.

Table 5. The comparision of the contents and percentage part of total protein between wall cell and protoplast of *S. cerevisiae* bakery strain from the control and experimental media

Type of medium/series	Contents of the total protein [g/100g d.w.]				Contents of the total protein [%]	
		Yeast walls				
	Yeast cell [g/100g d.w.yeast]	gd.w. wall	g/100g d.w. yeast	Protoplast [g/100g d.w. yeast]	Yeast cells	protoplast
YPD						
Series I	43.05	19.67	3.93	39.12	9.1	90.9
Series II	40.18	17.48	3.50	36.68	8.7	91.3
Series III	39.82	18.09	3.62	36.20	9.1	91.9
Means values	41.02c*	18.41	3.68a	37.34b	9.0	91.0
YPD+1.25g Mg^{2+}/dm^3						
Series I	57.70	51.19	4.24	53.46	7.3	92.7
Series II	53.98	18.95	3.79	50.19	7.0	93.0
Series III	54.53	19.60	3.92	50.61	7.2	92.8
Means values	55.40e	19.91	3.98a	51.42d	7.2	92.8
	*Means with the same le48tter did not differ significantly					

The yeasts grown in the experimental media were found to demonstrate a significantly higher mean content of total protein (55.4 g/100 g d.m of yeasts) than these cultured in the control media (41.0 g/100 g d.m. of yeasts). The addition of Mg^{2+} ions to the YPD medium had a beneficial effect on the biosynthesis of protein, which was proved by an approximately 35% increase in the protein content in the cells of the investigated yeast strain. The mean content of total protein in the cell walls of the yeasts from the control media reached 3.68 g of total protein

/ 100 g d.m. of yeasts while that reported in protoplasts accounted for 37.34 g of total protein / 100 g d.m., which constituted 9% and 91%, respectively, of the total protein in the cells. In the case of the yeasts obtained from the magnesium-enriched medium, the total protein content reached 3.98 g/100 g d.m. of yeasts in the cell walls and 51.42 g/100 g d.m. in the protoplasts (i.e. 7.2% and 92.8% of total protein in the cells, respectively). The differences observed between the total protein contents in the protoplasts from the control and experimental cultures were statistically significant, compared to the lack of significant differences between protein contents in the cell walls. The results obtained suggest that the addition of magnesium salt to the culture medium increased the total activity of the apparatus able to synthesise cellular proteins in the investigated strain of baker's yeasts.

In the protoplasts of the yeasts obtained from the experimental culture the content of magnesium (1.08 mg/g d.m. of yeasts) increased 3-fold when compared to the control culture medium (0.36 mg /g d.m. of yeasts), while the total protein content in the protoplasts increased by approximately 40% (from 37.34 g/100 g d.m. of yeasts to 51.42 g/100 g d.m.). These two facts incline to accept the preliminary hypothesis that under experimental conditions intracellular accumulation of magnesium might have led to the formation of metalloproteins described as bioplexes.

CONCLUSIONS

- Of the total pool of magnesium permanently bound with the cell of baker's yeasts *S. cerevisiae* No. *102* during a 24 h culture in the experimental medium (YPD + 1.25 g Mg^{2+}/dm^3), approximately 85% of magnesium ions were located in the cell wall.
- Enrichment of the cell biomass of *S. cerevisiae* No. *102* yeasts with magnesium in the experimental medium did not cause any change in the percentage distribution of this element neither in the cell nor in the protoplast, compared to the culture in the control medium. It may suggest that the dose of Mg^{2+} ions applied did not break the metabolic equilibrium of the cells.
- The 1.25 g Mg^{2+}/dm^3 dose of magnesium added to the experimental medium caused a significant increase the contents of magnesium in the cell wall and protoplast the cells of *S. cerevisiae* No. *102* yeasts, compared to the control culture.
- The 1.25 g Mg^{2+}/dm^3 dose of magnesium added to the experimental medium caused a significant increase the contents of the total protein in protoplast the cells of *S. cerevisiae* No. *102* yeasts, compared to the control culture.
- It seems that the extension of culture time of the investigated baker's yeast in the experimental medium over 24 h may cause transportation of a higher number of Mg^{2+} ions adsorbed on the cell wall surface during the logarithmic phase of growth to the inner part of the cell. These issues will be however elucidated in the further studies of the authors.

REFERENCES

1. Birch R. M., Walker G. M., 2000. Influence of magnesium ions on heat shock into S. cerevisiae cell by hypotonic downshift. Enzyme Microbiol. Technol. 26, 678-687.
2. Błażejak S., Duszkiewicz-Reinhard W., Gniewosz M., Rostkowska-Demner E., Domurad E.,2002. The study of *Saccharomyces cerevisiae* brewery yeast strain capacity of binding with magnesium in dynamic conditions. EJPAU Food Sci. Technol. 1(2).
3. Bryłka J., Więckowska E., Lewandowski W., Stępniak S., Bortnowska-Bareła B., 1995. Eksperymentalna chemia fizyczna [Experimental physical chemistry]. Wyd. SGGW Warsaw [in Polish].
4. Bui M. D., Gregan J., Jarosch E., Ragnini A., Schweyen R., J., 1999. The bacterial magnesium transporter CorA can functionally substitute for its putative homologue Mrs2p in the yeast inner mitochondrial membrane. J. Biol. Chem. 274, (29), 20438-20443.
5. Burbianka M., Pliszka A., 1983. Mikrobiologia żywności [Food microbiology]. PZWL Warsaw [in Polish].
6. Chmiel A., 1998. Biotechnologia. Podstawy mikrobiologiczne i biochemiczne [Biotechnology. Microbiology and biochemistry fundamental] PWN, Warsaw [in Polish].
7. Ciesarova Z., Smogrovicova D., Domeny Z., 1996. Enhancement of yeast ethanol tolerance by calcium and magnesium. Fol. Microbiol. 41 (6), 485-488
8. Duszkiewicz-Reinhard W., Gniewosz M., Błażejak St., Bańkowski A., 2002. Badanie zdolności wiązania magnezu przez drożdże piekarskie *Saccharomyces cerevisiae* w warunkach hodowli stacjonarnej [The study of *Saccharomyces cerevisiae* brewery yeast strain capacity of binding with magnesium in stationary conditions]. Acta Sci. Pol. Technol. Alim. 1(1), 17-26 [in Polish].
9. Emelyanowa E. V., 2001. Relationship between magnesium and iron uptake by the yeast *Candida ethanolica*. Proc. Biochem. 36, 517-523.
10. Graschopf A., Schtadler J. A., Hoellerer M., K., Eder S., Sieghardt M., 2001. The yeast plasma membrane protein Alr controls Mg^{2+} homeostasis and is subject to Mg^{2+} dependent control of its synthesis and degradation. J. Biol. Chem. 19 (276), 16216-16222.
11. Klepacka M., 2000. Analiza żywności, skrypt do ćwiczeńm [Food analysis]. Wyd. SGGW, Warsaw [in Polish].
12. Kurtzman C. P., Fell J. W., 1998. The yeast taxonomic study. Elsevier Sci., Amsterdam.

13. Lipke P. N., Ovalle R., 1998. Cell wall architecture in yeast: new structure and new challanges. J. Bacteriol. 180 (15), 3735-3740.
14. Lyticase L2524. 2002. Materiały firmy Sigma-Aldrich [Sigma Aldrich handbook]
15. MacDiarmid C. W., Gardner R. C., 1998. Overexpresion of the *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminium ion. J. Biol. Chem. 273 (3), 1727-1732.
16. Mardarowicz L., 1997. Drożdże w żywieniu drobiu [The yeast in poultry nourishment]. Pol. Drob. 9, 14-16 [in Polish].
17. Muller E., Loeffler W., 1987. Zarys mikologii dla przyrodników i lekarzy [Outline of micology for natural scientist and doctor]. PWRiL, Warsaw [in Polish].
18. Pasternakiewicz A., Tuszyński T., 1997. Effects of calcium, magnesium, cobalt (III) and zinc cations on the *Saccharomyces cerevisiae* growth. Pol. J. Food Nutr. Sci. 47 (4), 61-70.
19. Robinson R. K., Batt C. A.,Patel P. D., 2000. Encyclopedia of Food Microbiology. Academic Press, Oxford.
20. Sikorski Z., 2000. Chemia żywności: skład, przemiany i właściwości żywności [Food chemistry: composition, changes and properties of food]. WNT, Warsaw [in Polish].
21. Soral-Śmietana M., Wronkowska M., Świgoń A., Kłębukowska L., 1999. Biopierwiastki - możliwości tworzenia kompleksów z polimerami organicznymi [Biochemical elements- possibility to create of complex with organic polymers]. Biul. Magnezol. 4 (2), 418-423.
22. Suizu T. Tsutsumi H., Kawado A., Imayasu S., Inose T., Kimura A., Murata K., 1994. Induction of yeast sporulation by lysine – related compounds and glutathione – rich conditions. J. Ferm. Bioeng. 77 (5), 568–572.
23. Tuszyński T., Pasternakiewicz A., 2000. Bioaccumulation of metal ions by yeast cell of *Saccharomyces cerevisiae*. Pol. J. Food Nutr. Sci. 4, 31–39.
24. Vandergrift B., 1991. Bioplexes: Practical application. Biotechnology in the feed industry. Proc. Alltech's Seventh Annual Symp.
25. Walker G. M., 1994. The roles of magnesium in biotechnology. Crit. Rev. Biotechnol. 14 (4), 311-354.

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