*Acta Alimentaria, Vol. 31 (4), pp. 393–405 (2002)*

# YEAST CELLS AS SOURCES OF ESSENTIAL MICROELEMENTS AND VITAMINS $B_1$ AND $B_2$

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(Received: 6 July 2001; accepted: 11 February 2002)

Baker's yeast (*Saccharomyces cerevisiae*-Sz1) enriched in chromium, iron, selenium or zinc was prepared by shaken cultivation and laboratory fermentation. Determination of the cellular distribution of microelements indicated that a considerable portion (68–88%) was bound to the cell constituents, a very little part was solved only in the cytosol and vacuole. More than half of the original vitamins B content has been lost during the general guarantee time (12 months) and the microelements had only little influence on it. Enrichment of yeast cells with iron was accompanied by considerable increase in vitamin $B_2$ content. Ascorbic acid as an antioxidant additive decomposed very rapidly during storage, while tocopherol proved quite stable in the non-enriched yeast prep. Selenium enrichment did not affect the inactivation of ascorbic acid, while it accelerated the decomposition of tocopherol significantly.

**Keywords:** yeast, microelements, vitamins $B_1$ and $B_2$, ascorbic acid, tocopherol

Microelements and vitamins play a vital role in several human metabolic processes, which are needed in relatively small amounts by the human body to perform highly specific metabolism, growth and maintenance of normal cell and organ functions. They are essential food factors of very high biological activity (BASU & DICKERSON, 1996).

For satisfactory functions of the body, it is also necessary to maintain a suitable microelement level. Zinc, iron, chromium and selenium are in a distinguished position. They have a role in many functions of the human body, e.g. zinc in growth, tasting, synthesis of blood cells, blood coagulation, lipid and protein metabolism, cerebral activity (BARCELOUX, 1999; CUNNINGHAM-RUNDLES, 1990; NISHI, 1996). Iron is vital for all the living beings, in the synthesis of blood cells and the cerebral activity (COOK, 1990; CRICHTON & WARD, 1992), the chromium in glucose, lipid and protein metabolism (ANDERSON, 1989; ANDERSON & POLANSKY, 1991; DUBOIS & BELLEVILLE, 1991; MORRIS & BLUMSOHN, 1992), while selenium in growth, activation of the immune system and in the regeneration of liver (DANIELS, 1996). It is also known that selenium constitutes an essential component of the enzyme glutathione peroxidase in the

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form of selenocysteine (AHRENS, 1997). Selenium can also protect against the negative effects of free radicals and acid metabolites (ALAEJOS & ROMERO, 2000).

Vitamin and microelement deficiency in modern human nutrition is a big problem. There are several attempts to improve the microelement content of food in order to develop functional foods. The continuous trace element intake is an important factor in keeping the good health condition (ANON, 1996). In the form of inorganic salts, microelements are usually absorbed in the human intestine rather insufficiently. Yeast biomass enriched in microelements (e.g. iron, chromium, selenium, zinc) can be a new natural microelement source in human and animal nutrition, in the form of various medicinal preparations (KORHOLA & EDELMAN, 1986; JANZSÓ et al., 1995).

Yeast cells are able to take up most of the microelements and incorporate them into organic molecules, which are more favourable to both humans and animals than inorganic compounds.

Metal accumulation by yeast cells involves a combination of extracellular accumulation and transport mechanisms. The first stage termed biosorption, usually referred to as passive binding, is an initial rapid and reversible accumulation stage. The stability of heavy metals bound to the surface of yeast cells depends on pH and the presence of competing cations and organic complexing ligands. The second stage, usually referred to as active transport, is a slower intracellular bioaccumulation, which is often irreversible and is related to the metabolic activity of the cells (SHUMATE & STRANDBERG, 1985).

There is little detailed work pertaining to *Saccharomyces cerevisiae* for divalent cation uptake in general, so a coherent picture is not available. This is in part due to the many factors that can affect the kinetics of divalent cation uptake in yeast, such as surface potential effects (ROOMANS et al., 1979; BORST-PAUWELS & THEUVENET, 1984), the possible existence of multiple transport systems (NORRIS & KELLY, 1977; BORST-PAUWELS, 1984), toxicity and other factors pertaining to the physiological state of the cells and varying external conditions (GADD, 1988).

The intracellular form of iron in yeast cells is $Fe^{2+}$. Cells are able, however, to utilise the $Fe^{3+}$ ion content of the culture media, too. In this case a ferri-ion reducing enzyme system is a prerequisite, which reduces the $Fe^{3+}$ to $Fe^{2+}$ before being transported. CARANE and co-workers (1982) as well as YAMASHOJI and KAIMOTO (1986) found an extracellular ferri-cyanide reducing system in *Saccharomyces cerevisiae*. Later, LESUISSE and co-workers (1987, 1990) proved the presence of a transmembrane redox system. There are two transport systems existing for the uptake of iron in *Saccharomyces cerevisiae*. The high affinity system ($K_m$ = 0.15 μmol) is induced under iron limitation and requires the activity of the Fet3p product of *FET3* gene (EIDE & GUARENTE, 1992; ASKWITH et al., 1994). The second iron transport system with lower substrate affinity ($K_m$ = 30 μmol) is working in iron replete cells and uses the Fet4p, which is the product of *FET4* gene (DIX et al., 1994). The *fet3 fet4* double mutant, however, remained viable, while they were sensitive to the iron limitation,

indicating the presence of a third transport system in the cell. SPIZZO and BYERSDORFER (1997) identified the *FET5* gene, which encodes the multicopper oxidase, closely related to Fet3p, both playing roles in the iron transport and homeostasis.

The concentration of iron, required by the yeast cells under normal physiological conditions is quite low, the optimum concentration is between 1–10 μmol. Intracellular content of iron can be different, depending on several environmental conditions. Certain enzymes are especially sensitive for the inhibitory effect of iron (e.g. malate, pyruvate and succinate dehydrogenases). The toxic effect can also be attributed to iron permeability (JONES & GREENFIELD, 1984).

Selenium-enriched yeast biomass is currently used as a source of selenium in nutritional supplementation, and its consumption has been found to be associated with statistically significant reduction in total cancer incidence and cancer mortality (ALAEJOS & ROMERO, 2000; ANON, 1996).

Yeast and dried yeast are excellent sources of vitamins B (LIST & HÖRHAMMER, 1973), which are water-soluble and are not stored for a long time in the body. These vitamins serve as coenzymes for lipid, carbohydrate and protein metabolism. Therefore their supply must be regular in the diet (LAWRENCE & CARL, 1996).

It has been shown in many cases that the stability of certain vitamins is widely influenced by the concentration of various metal ions, but the effect of the enrichment of yeast with microelements on their endogenous vitamin B content has not been reported yet.

Vitamin C is an essential micronutrient required for an array of biological functions, including enzymatic reactions and antioxidation. It is the most important antioxidant in the body. Vitamin E has also an important antioxidative and protective function (BASU & DICKERSON, 1996). Vitamin C or vitamin E, together with selenium, have considerable antioxidant effect. Synergism of selenium and vitamin E has been proved in many cases (BARTFAY et al., 1998; GIRODON et al., 1999). Therefore, selenium-enriched yeast biomass is occasionally supplemented with vitamin E.

The aim of our research was to study the cellular distribution of iron, zinc, chromium and selenium in yeast cells and to draw conclusion about the stability of the endogenous vitamins B in yeast cells enriched with microelements and that of the ascorbic acid and tocopherol used as antioxidants in selenium-enriched yeast products.

## 1. Materials and methods

### *1.1. Strain*

Commercial baker's yeast strain *Saccharomyces cerevisiae* Sz-1.

### *1.2. Culture medium*

Yeast extract-pepton-saccharose (YEPS) broth: 5 g $l^{-1}$ yeast extract (Oxoid), 5 g $l^{-1}$ pepton (Oxoid), 30 g $l^{-1}$ saccharose (pH 6).

*1.3. Supplementation with microelements*

Yeast suspensions contained the microelements in the following concentrations: $CrCl_3 \cdot 6H_2O$: 25–100 μg $ml^{-1}$, Fe(III)-ammonium-citrate: 125–500 μg $ml^{-1}$, $Na_2SeO_3$: 43–700 mg $ml^{-1}$, $ZnSO_4 \cdot 7H_2O$: 36–288 μg $ml^{-1}$.

*1.4. Enrichment of yeast cells with microelements*

*1.4.1. Shaken cultures*. Hundred ml YEPS medium in 300 ml Erlenmeyer flask was inoculated with 24 h-old culture of baker's yeast in the concentration of $10^6$ cells $ml^{-1}$. Microelements were added in the form of soluble salts at the final concentrations indicated in 1.3. The cultures were shaken at 200 r.p.m. at 28 °C until the cells entered the stationary phase of growth.

*1.4.2. Laboratory scale fermentation.* Cultivation was performed in a 2 l fermenter (Bioflo 3, New Brunshwick). Culture medium and inoculation were the same as in shaken cultures. Fermentation conditions: aeration 1 v/v/m, stirring by 200 r.p.m., temperature: 28 °C. Fermentation was finished when the cells entered into the stationary phase of growth.

*1.5. Determination of cellular distribution of microelements*

Cells were harvested by centrifugation and washed twice with deionized water. Cell wall bound cations were mobilised by washing the cells with 50 mmol EDTA-$Na_2$ (pH 7.0). Intracellular cations were differentially extracted from the cells by using DEAE-dextran according to the method described by WHITE & GADD (1987). Complete permeabilisation of the cytoplasmic membrane was checked by staining the cells with malachite-green dye.

The extracted microelement fractions were as follows (WHITE & GADD, 1987):

- *cell wall bound fraction*: cell extract obtained after EDTA-$Na_2$ treatment
- *soluble cytoplasmic fraction:* cell extract obtained after treatment with sorbitol-DEAE-dextran
- *soluble vacuolar fraction:* cell extract obtained after 60% methanol treatment
- *bound to cell constituents:* cell sediments obtained after 60% methanol treatment

*1.6. Inactivation of cellular biomass*

Cells were harvested by centrifugation, washed twice with water, dried by liophylisation and inactivated by heating at 80 °C for 2 h. Vitamins were added to the inactivated cells as solutions, and the cells were dried again at 50 °C for 1 h. The vitamin C and vitamin E (tocopherol acetate), added to yeast biomass, were 167 and 15 mg $g^{-1}$ dry weight, respectively. Samples were stored in a refrigerator at 4 °C.

*1.7. Measurement of vitamins B concentration*

Vitamins $B_1$ (thiamine) and $B_2$ (riboflavin) content of the yeast biomass was determined with a microbiological analytical method both in viable cells and after thermal inactivation by the method of GYÖRGY and PEARSON (1967). For thiamine, *Lactobacillus fermentum* strain ATCC 9338 and for riboflavin, *Lactobacillus casei* strain ATCC 7469 was used.

*1.8. Measurement of microelement contents*

Concentrations of Cr, Fe, Se and Zn were determined by inductively coupled plasma - atomic emission spectroscopy (ICP-AES) model Thermo Jarrel Ash-ICAP 61.

*1.9. Measurement of vitamin C and E contents*

Determination of vitamin C (ascorbic acid) concentration was performed by high performance liquid chromatography (HPLC, Beckman series), consisting of a Model 165 variable wavelength UV/visible detector. Detector signals were recorded by a Model C-R2A Shimadzu integrator. To record spectrum of ascorbic acid, a Waters model 990 photodiode-array detector was used. The range of detection wavelength was 190–340 nm. The isocratic mobile phase was prepared by adding 1.0 ml of 20% tetrabutyl ammonium hydroxide and 30 ml of methanol to 970 ml of 0.01M potassium dihydrogen phosphate solution. The pH was adjusted to 2.75 using 85% phosphoric acid. The flow rate was 1.0 ml $min^{-1}$. The separation was performed on a column (25 cm length × 4.6 mm i.d.) packed with ODS-2, 10 μm. The injection volume was 20 μl. The chromatographic peak of the reduced form of ascorbic acid was identified by comparing both retention time and absorbance spectrum with that of the standard material.

Vitamin E (tocopherol) was determined using a Beckman Model 114 M isocratic pump, a Shimadzu fluorometric detector, and Shimadzu C-R3A or Waters-740 Data Model integrators. The separation of tocopherol was performed on a column (25 cm length × 4.6 mm i.d.) packed with Si-100-S, 10 μm. The mobile phase was n-hexane–ethanol (99.5:0.5) with a flow rate of 1.2 ml $min^{-1}$. The detection wavelengths used for excitation and emission were 295 nm and 320 nm, respectively. For peak identification, retention time and maximum absorption spectra of tocopherol were compared with those of standard materials, which were also used for quantification.

## 2. Results and discussion

*2.1 Production of yeast-cell biomass enriched with microelements chromium, iron, zinc and selenium*

For the enrichment of microelements in yeast cells, the application of much higher concentration of the microelements in the culture media than under normal

physiological growth conditions are necessary (KORHOLA & EDELMAN, 1986; JANZSÓ et al., 1995). These microelements can be, however, more or less toxic for the cells in high concentrations. According to our results obtained, iron was not toxic even at the highest concentration applied, while zinc, chromium and selenium compounds inhibited the growth of cells to a different extent (Table 1). We applied these microelements below the MIC values, which permitted biomass production similar to the control (i.e. cultures without added microelements). Data concerning the enrichment in shaken cultures (100 ml) and in laboratory fermenter (2 l) are shown in Table 1. A little higher cellular concentration was obtained for Zn and Se in shaken cultures than in laboratory fermenter. This difference was even more pronounced in the case of Cr and Fe in favour of the shaken cultures.

*Table 1.* Inhibitory effect of microelements for yeast and cellular enrichment in shaken and fermenter cultures

| Microelement source | MIC[a] value ($\mu g\ ml^{-1}$) | Concentration for enrichment ($\mu g\ ml^{-1}$) | Concentration of microelements in yeast biomass ($\mu g\ g^{-1}$ d.w.[b]) | |
|---|---|---|---|---|
| | | | Shaken culture | Fermenter culture |
| $Zn\ SO_4 \cdot 7\ H_2O$ | 288 | 50 | 1538 | 1075 |
| $Cr\ Cl_3 \cdot 6\ H_2O$ | 250 | 50 | 28 | 4 |
| Fe (III)-ammonium-citrate | >500 | 500 | 3688 | 104 |
| $Na_2SeO_3$ | 346 | 43 | 2875 | 2159 |

[a] MIC: minimal inhibitory concentration
[b] d.w.: dry weight

### *2.2. Cellular distribution of microelements*

Fractions of microelements localised in different yeast cell constituents were determined by differential extraction, using subsequent permeabilisation of cytoplasmic and vacuolar organelle membranes (WHITE & GADD, 1987). Results are shown in Table 2. The EDTA-chelated microelement content of the cell wall was between 2 and 11% of the total microelement concentration. Cytosol contained a very low portion of microelements (1%), only selenium content was considerable (13%). Soluble microelement fraction of the vacuoles (more precisely that of the compartments) was again low, the selenium content was relatively the highest (17%) in this case, too. We considered the remained microelement content as bound fraction, supposing that most part of it was bound to the organic molecules of the intracellular structures, mainly to proteins and carbohydrates. We cannot exclude, however, that a part of this bound fraction was localised in the cell wall as EDTA non-mobilised microelement content. The bound fraction was between 68 and 88% of the total microelement concentration.

*Table 2.* Cellular distribution of chromium, iron, selenium and zinc in yeast cells enriched with these microelements in shaken cultures

| Yeast products | Concentration adsorbed by the cell wall | | Concentration in the cytosol | | Concentration in the compartments | | Bound concentration | | Total concentration | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ($\mu g\ g^{-1}$ d.w.) | (%) | ($\mu g\ g^{-1}$ d.w.) | (%) | ($\mu g\ g^{-1}$ d.w.) | (%) | ($\mu g\ g^{-1}$ d.w.) | (%) | ($\mu g\ g^{-1}$ d.w.) | (%) |
| Yeast enriched with chromium (50 $\mu g\ ml^{-1}$) | 1 ± 1 | 2 | u.d.l. | u.d.l. | 3 ± 1 | 10 | 26 ± 4 | 88 | 30 ± 3 | 100 |
| Yeast enriched with iron (500 $\mu g\ ml^{-1}$) | 400 ± 25 | 11 | 35 ± 3 | 1 | 29 ± 2 | 1 | 3238 ± 32 | 87 | 3702 ± 29 | 100 |
| Yeast enriched with zinc (50 $\mu g\ ml^{-1}$) | 150 ± 19 | 10 | 6 ± 2 | 1 | 136 ± 14 | 8 | 1277 ± 18 | 81 | 1569 ± 22 | 100 |
| Yeast enriched with selenium (346 $\mu g\ ml^{-1}$) | 43 ± 8 | 2 | 387 ± 22 | 13 | 483 ± 11 | 17 | 2001 ± 41 | 68 | 2914 ± 37 | 100 |

u.d. l.: under detection limit
± standard error
Values are the results of three independent experiments

*Table 3.* Vitamins $B_1$ and $B_2$ content of the yeast biomass enriched with different microelements

| Samples | Vitamin content of raw yeast | | Vitamin content of inactivated yeast | | Decrease of vitamin content during inactivation (%) | |
|---|---|---|---|---|---|---|
| | $B_1$ ($\mu g\ g^{-1}$ d.w.) | $B_2$ ($\mu g\ g^{-1}$ d.w.) | $B_1$ ($\mu g\ g^{-1}$ d.w.) | $B_2$ ($\mu g\ g^{-1}$ d.w.) | $B_1$ | $B_2$ |
| Control yeast | 66 ± 21.01 | 50 ± 7.26 | 51 ± 8.61 | 21 ± 6.70 | 23 | 58 |
| Yeast enriched with chromium ($4\ \mu g\ g^{-1}$ d.w.) | 71 ± 36.93 | 60 ± 28.71 | 45 ± 13.32 | 28 ± 3.64 | 52 | 48 |
| Yeast enriched with iron ($104\ \mu g\ g^{-1}$ d.w.) | 66 ± 26.40 | 179 ± 38.7 | 45 ± 12.70 | 34 ± 5.39 | 31 | 81 |
| Yeast enriched with zinc ($1075\ \mu g\ g^{-1}$ d.w.) | 71 ± 32.67 | 60 ± 27.01 | 63 ± 28.22 | 31 ± 8.75 | 11 | 48 |
| Yeast enriched with selenium ($2159\ \mu g\ g^{-1}$ d.w.) | 98 ± 29.73 | 100 ± 53.20 | 45 ± 12.23 | 33 ± 7.94 | 54 | 67 |

± standard error

Values are the results of three independent experiments with 4 replicates

*Table 4.* Vitamins $B_1$ and $B_2$ content of the inactivated yeast biomass enriched with different microelements after 6 and 12 months

| Samples | Vitamin content after 6 months storage | | Vitamin content after 12 months storage | | Decrease of vitamin content (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | $B_1$ ($\mu g\ g^{-1}$ d.w.) | $B_2$ ($\mu g\ g^{-1}$ d.w.) | $B_1$ ($\mu g\ g^{-1}$ d.w.) | $B_2$ ($\mu g\ g^{-1}$ d.w.) | After 6 months | | After 12 months | |
| | | | | | $B_1$ | $B_2$ | $B_1$ | $B_2$ |
| Control yeast | 41 ± 6.23 | 20 ± 4.82 | 20 ± 2.99 | 9 ± 0.94 | 20 | 5 | 60 | 61 |
| Yeast enriched with chromium (4 $\mu g\ g^{-1}$ d.w.) | 42 ± 11.41 | 15 ± 6.34 | 19 ± 12.31 | 13 ± 3.92 | 13 | 46 | 60 | 64 |
| Yeast enriched with iron (104 $\mu g\ g^{-1}$ d.w.) | 29 ± 8.88 | 20 ± 5.55 | 14 ± 5.13 | 13 ± 3.09 | 36 | 41 | 68 | 62 |
| Yeast enriched with zinc (1075 $\mu g\ g^{-1}$ d.w.) | 35 ± 8.00 | 16 ± 5.82 | 23 ± 5.53 | 14 ± 5.55 | 44 | 48 | 63 | 55 |
| Yeast enriched with selenium (2159 $\mu g\ g^{-1}$ d.w.) | 33 ± 7.21 | 17 ± 5.23 | 20 ± 5.23 | 17 ± 2.88 | 27 | 48 | 55 | 48 |

± standard error

*2.3. Vitamins B of raw and inactivated yeast biomass*

Vitamin $B_1$ and $B_2$ contents of raw and thermal inactivated yeast biomass, produced in a fermenter, are shown in Table 3. The control yeast (cultivated without microelement supplementation) contained relatively high vitamin B amount, confirming the general view that yeast biomass is a good source of vitamins B in nutrition (LIST & HÖRHAMMER, 1973). The actual vitamin contents, however, vary widely, depending mainly on the nature and composition of the propagation medium (HARRISON, 1993). Thermal inactivation decreased the vitamin content for both vitamins B, as expected. Vitamin $B_1$ proved more stable, the decrease was only 23%. Loss of vitamin $B_2$ content, however, was considerable (58%). Enrichment of yeast cells with microelements did not have a big influence on the vitamin B content of cells with one exception: the iron-enriched cells contained about 3 times more vitamin $B_2$ than the control yeast. Microelement content of the cells did not affect thermal stability of vitamin $B_1$, nor that of the vitamin $B_2$ with one exception: the high vitamin $B_2$ content of iron enriched cells decreased to the mean level of the other yeast samples. Further decrease in both vitamins content was slow during the first 6 months of storage in the control yeast, but decomposition accelerated in the next 6 months (Table 4). Stability of vitamins $B_1$ and $B_2$ was different in the microelement enriched yeast biomass, but no general rule could be concluded concerning the specificity of microelement content after 6 months storage. Vitamin content decreased to similar extent in the case of control and enriched yeasts during the next 6 months storage, except for the selenium-enriched yeast, where no further decrease in vitamin $B_2$ content was found.

*2.4. Decomposition of antioxidant additives during storage of inactivated yeast biomass*

Antioxidants, especially tocopherol are frequently used for enhancing the physiological effect of selenium (BARTFAY et al., 1998). We measured the chemical stability of antioxidants, ascorbic acid and tocopherol during 12 months storage of control and selenium-enriched yeast biomass. The reason was that the suggested storage period of inactivated yeast preps and vitamins is 12 months. Data are shown in Table 5. Ascorbic acid almost completely decomposed during 6 months storage at 4 °C, and the residual ascorbic acid content decreased below the detection limit after 12 months storage. Selenium content of the biomass did not influence the rapid decomposition of ascorbic acid. Therefore, addition of ascorbic acid to the selenium-enriched inactivated yeast biomass is not recommended.

Chemical stability of tocopherol proved quite good in the case of control yeast prep during the first 6 months storage. Decrease was 27.8 % only, but we measured a rapid, almost complete decomposition during the next 6 months. Selenium enrichment considerably accelerated decomposition, the original tocopherol content decreased by 76.3% for the end of 6 months storage and we could detect only a negligible amount at the end of 12 months storage. According to the results obtained, the guarantee time of the non-enriched, tocopherol supplemented yeast prep can be maximised in 6 months.

*Table 5.* Changes of added antioxidant contents during storage of inactivated yeast biomass

| Antioxidants | Storage period (months) | Concentration of antioxidants in the samples | | | |
|---|---|---|---|---|---|
| | | Control yeast | | Selenium-enriched yeast | |
| | | Total (mg $g^{-1}$ d.w.) | Loss (%) | Total (mg/g d.w.) | Loss (%) |
| Ascorbic acid | 0 | 121.750 | – | 118.232 | – |
| | 6 | 1.564 | 98.7 | 1.603 | 98.7 |
| | 12 | 0 | 100.0 | 0 | 100.0 |
| Tocopherol acetate | 0 | 49.425 | – | 38.829 | – |
| | 6 | 35.689 | 27.8 | 9.213 | 76.3 |
| | 12 | 1.455 | 97.1 | 0.589 | 98.5 |

After this period the prep will loose the dual physiological effects of the active ingredients. More considerable decrease of the tocopherol content in the case of selenium-enriched yeast biomass can be explained by the strong oxidising effect of selenium toward tocopherol (BARTFAY et al., 1998). However, this result is a rapid inactivation of tocopherol, which requires additional supplementation of tocopherol as physiologically active ingredient in a selenium diet.

## 3. Conclusions

Yeast cells, enriched in chromium, iron, selenium or zinc contained these microelements mainly in the form of undissolved, bound compounds. Enrichment of yeast cells with iron was accompanied by considerable increase in vitamin $B_2$ content, but enrichment with other microelements did not influence the vitamins $B_1$ and $B_2$ content significantly.

More than half of the original vitamins $B_1$ and $B_2$ content of the cells has been lost during the general guarantee time (12 months) of the medicinal yeast preps, the microelements had only little influence on it.

Ascorbic acid as an antioxidant additive decomposed very rapidly during storage, while tocopherol proved quite stable in the non-enriched yeast prep. Selenium enrichment did not affect the inactivation of ascorbic acid, while it accelerated the decomposition of tocopherol significantly.

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The authors wish to express their gratitude to J. REZESSY-SZABÓ for her help in fermentation experiments, P. FODOR for providing the ICP possibilities and H. DAOOD for the HPLC measurements. E. VARGA was the recipient of short time doctoral fellowships from the Hungarian Academy of Sciences and the Hungarian Ministry of Education, which is highly appreciated.

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