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THE EFFECTS OF ZIRCONIUM, A LESS KNOWN MICROELEMENT, ON BASIC FERMENTATION CHARACTERISTICS AND PROTEIN COMPOSITION OF *SACCHAROMYCES CEREVISIAE*

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A strain of *Saccharomyces cerevisiae* used in the food industry was propagated in the presence of zirconium ascorbate or zirconium citrate complex. The propagation of the yeast was slightly inhibited by zirconium (Zr) in a complex form, but it was not toxic. The Zr content in the fermentation medium decreased faster when using ascorbate complex than the citrate complex. The ascorbate complex was better accumulated by the yeast (4300 μg Zr g^{-1} dry mass) than the citrate complex (1600 μg Zr g^{-1} dry mass). The total amino acid content of the yeast cells decreased in the presence of both complexes. The concentration of some amino acids [threonine (Thr), proline (Pro), phenylalanine and cysteine (Cys)] was increased by 10–39% in the medium containing Zr ascorbate, while that of other amino acids [arginine (Arg), serine (Ser), methionine (Met) and glutamic acid (Glu)] decreased by 18–60%. As a result of the presence of zirconium citrate the concentration of Glu, aspartic acid (Asp), leucine (Leu), Thr, valine (Val), Ser, Arg, Pro and Met decreased by 19–32%, and the concentration of Cys increased by 59%.

Keywords: *Saccharomyces cerevisiae*, zirconium ascorbate, zirconium citrate, zirconium accumulation, amino acid concentration

Thirty-one elements have already been proved to have essential or beneficial physiological effect on the metabolism of plants, animals and prokaryotes but possible physiological effect of further 34 elements is supposed (MERTZ, 1993; PAIS, 1996). Zirconium (Zr) is one of the latter group and its certain biological effects were examined in the present experiment.

We do not know much about Zr as a microelement, however its fungicidal, bactericidal and enzyme inhibitory effect was examined using inorganic Zr compounds (SCHARRER, 1955; RÜTTNER et al., 1987; COUTURE et al., 1989). Two-hour-long binding of human serum apotransferrin to Zr(IV) in the presence of nitrilotriacetate, citrate or ethylendiaminetetraacetate (EDTA) as donor ligands was described but its biological importance is not known (ZHONG et al., 2002).

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Since the knowledge on the biological activity of Zr is limited (KÖRÖS, 1980), our aim was to examine the effect of Zr with ascorbic acid and citric acid ligands on the growth rate, Zr-accumulation and amino acid content of *Saccharomyces cerevisiae*. Previous results (HEGÓCZKI et al., 1995, 1997) proved that *S. cerevisiae* accumulates different trace elements (Ti, Cr, etc.), it can be used for the examination of their biological effects, and it can function as a suitable eukaryotic model. The biological effect of Zr on *S. cerevisiae* is of interest because this yeast species is widely used in the human alimentation, too.

1. Materials and methods

1.1. Chemicals

Zr was provided in the form of complexes. Zirconyl chloride ($ZrOCl_2 \cdot 8H_2O$) was the basic compound and L-ascorbic acid and citric acid were applied as ligands. Concentrated hydrochloric acid was used to the complex formation. The pH was adjusted with the addition of KOH solution (10% w/w). All reagents were of analytical grade. The complex formation was monitored by photometric method.

1.2. Instrumentation

Spectrophotometric measurements were carried out with a PC-controlled GBC 916 UV/VIS spectrophotometer (GBC, Australia) equipped with 10 mm quartz cuvettes. The acid concentration of the blank solution and the complex was adjusted to the same value. The complex was filtered on nitrocellulose filter (pore size 0.22 μm).

The measurements were done with automatic acid analyser (Aminochrom II. OE-914, Labor MIM, Budapest, Hungary) at optimal parameters (SUHAJDA et al., 2000).

1.3. Yeast strain

The *S. cerevisiae* strain (Y00891) used in the present examination was provided by National Collection of Agricultural and Industrial Microorganisms, Szent István University (NCAIM). It was cultured in NCAIM 0001 medium (malt extract 2%, agar 2%) at 26 °C for 48 h.

1.4. Fermentation

The fermentation medium and the circumstances of the fermentation were the same as described by SUHAJDA and co-workers (2000). The experiments were carried out in three fermentation media, (i) nutrient broth (basic), (ii) basic medium with the addition of ascorbic acid or citric acid and (iii) basic medium with the addition of Zr ascorbate or Zr citrate. The filtered Zr complexes were given to the fermentation media in five equal doses. The final concentration was 100 mg Zr l^{-1} in the case of both complexes.

The fermentation media were inoculated with 10^9 colony forming units of *S. cerevisiae* and incubated at 26 °C for 24 h. The Zr concentration of the fermentation media was measured continuously without sample preparation. Separation of the biomass from the culture medium was carried out by centrifugation for 20 min at 3000×*g*. It was then washed three times in deionized water to remove unbound Zr and treated with acetone. After this treatment the biomass was precipitated and it was filtered in a stainless steel vacuum filtration system (500 ml, ø 47/50 mm, pore size 1.2 μm, Schleicher and Schuell). After filtration the yeast was dried at room temperature and analysed.

Propagation of yeast cells was determined by measuring the optical density. The absorbance was measured at 600 nm, and the control solution was sterile fermentation medium.

1.5. Analytical process

1.5.1. Glucose concentration. Glucose concentration of the fermentation medium was determined by the Schoorl's reduction method (ERDEY, 1956).

1.5.2. Zr concentration. Zr concentration of the fermentation medium and the biomass were measured using Arsenazo III reagent as described previously (SAVVIN, 1961; SNELL, 1978). The biomass was treated in two steps prior to analysis. In the first step the samples were left at room temperature for 24 h in the mixture of 2 ml concentrated nitric acid and 2 ml hydrogen peroxide. In the second step the sample was heated in a closed Teflon bomb at 110 °C,for 40 min. During the digestion, reductive nitrous gases are formed and they attack the reagent Arsenazo III. To prevent this negative effect, the digested samples were dehydrated with infrared lamp and deionized water was added to get 10 ml of solution. The sample was measured at 665 nm. The results are the average of three replicate determinations.

1.5.3. Amino acid concentration. The sample preparation for the analysis of the amino acid of the yeast cells was carried out as described by other authors (SIMON-SARKADI & SZERZŐ, 1985; TÖMÖSKÖZI et al., 1993).

2. Results and discussion

2.1. Investigation of zirconium complexes

Ascorbic acid and citric acid were used in the production of complexes. The metal-ligand ratio was 1:10 in the case of both compounds. The new structure was evident in the UV spectrum (Fig. 1). The development of the complex was a time reaction; it reached its maximum within 96 h, so no further change was seen in the UV spectrum on the fifth day (data not shown).

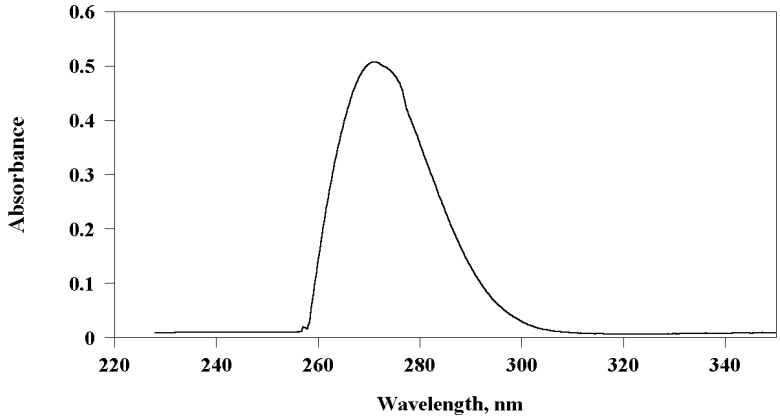

Fig. 1. Spectrum of Zr ascorbate (0.5 μg Zr ml^{-1})

2.2. Effect of the Zr complexes on the propagation of the S. cerevisiae

Both ascorbic acid and Zr ascorbate slightly decreased the propagation rate of *S. cerevisiae* in the first 7 h of incubation (Figs 2, 3). Addition of citric acid and Zr citrate had no similar effect. Ascorbic acid after the 7th h and citric acid after the 5th h increased yeast cell production compared to basic nutrient broth, while addition of Zr-complexes decreased the propagation rate. The OD of the fermentation medium was in the 24th h about 1.2-times higher in the presence of Zr ascorbate than in the presence of Zr citrate.

In the cell mass production there was no significant difference detected in the presence of Zr ascorbate or Zr citrate (5.4 g l^{-1} and 5.7 g l^{-1}), while the cell mass was 4.6 g l^{-1} in the basic medium.

The decrease of the glucose in the fermentation media is shown in Figs 4 and 5.

No significant difference was seen in the glucose utilisation in the presence of the two compounds.

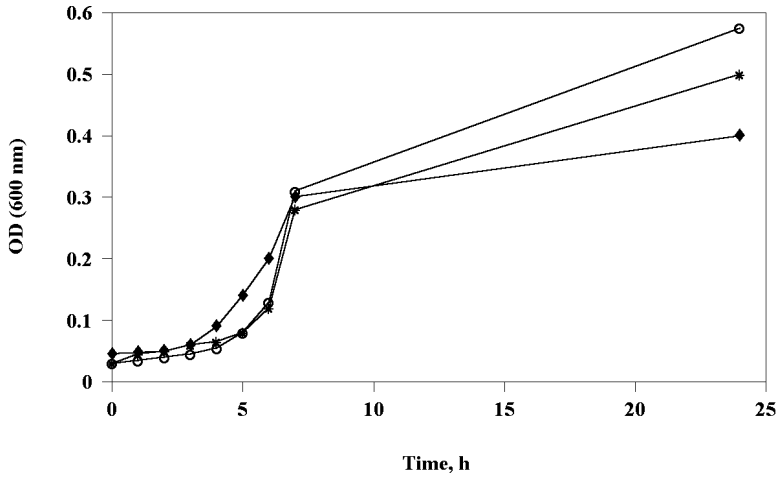

Fig. 2. Effect of ascorbic acid and Zr ascorbate on propagation of *S. cerevisiae.*
○: Basic; ✻: basic + ascorbic acid; ♦: basic + Zr ascorbate

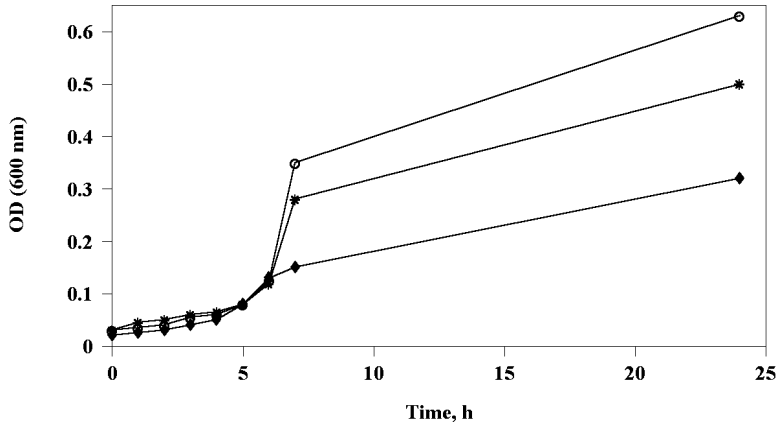

Fig. 3. Effect of citric acid and Zr citrate on propagation of *S. cerevisiae.*
○: Basic; ✻: basic + citric acid; ♦: basic + Zr citrate

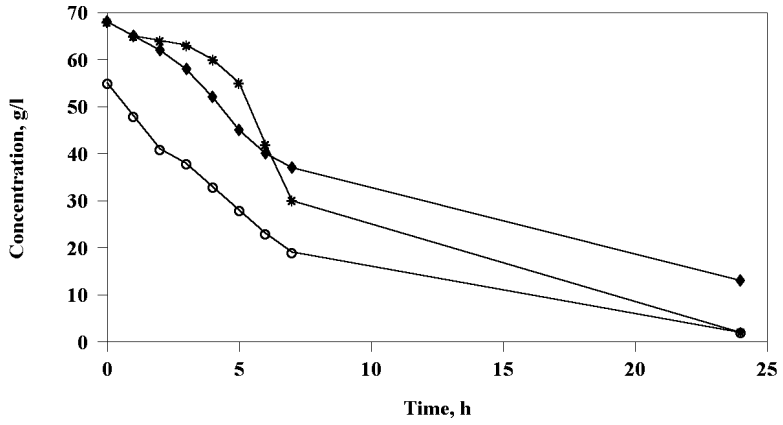

Fig. 4. Glucose concentration of the fermentation media.
○: Basic; ✻: basic + ascorbic acid; ♦: basic + Zr ascorbate

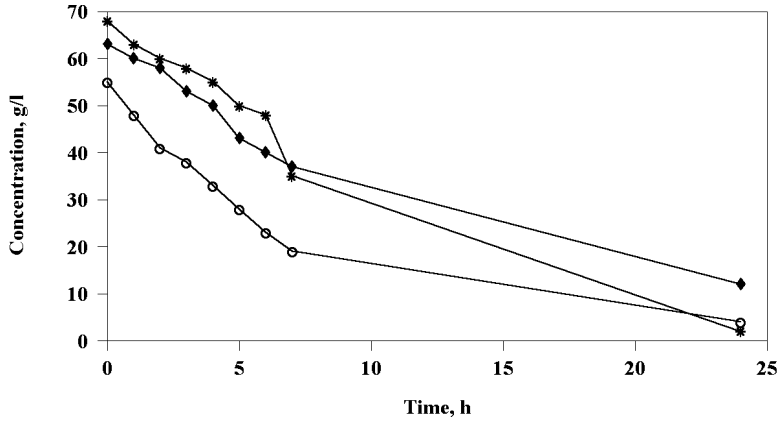

Fig. 5. Glucose concentration of the fermentation media.
○: Basic; ✻: basic + citric acid; ♦: basic + Zr citrate

2.3. Zr content of the S. cerevisiae

The concentration of ascorbate complex decreased fast compared to the theoretical value. After completing the dosage of Zr 56% and after 24 h 28% of the total amount of Zr could be detected in the fermentation medium. The utilisation of the citrate complex was much slower (Fig. 6).

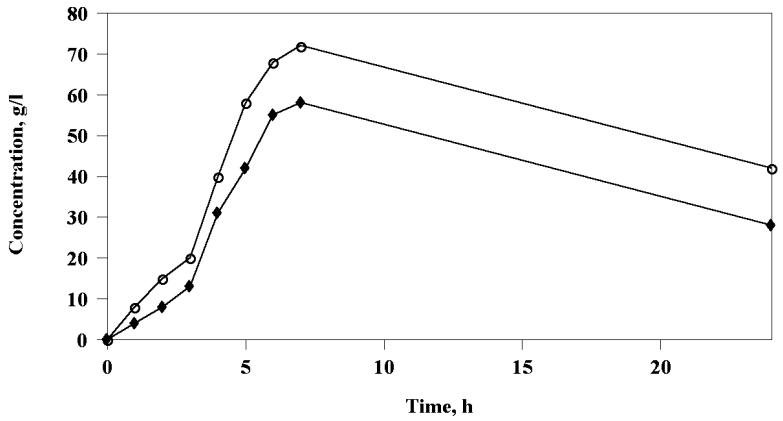

Fig. 6. Zr content in the fermentation medium.
○: Basic medium + Zr citrate; ♦: Basic medium + Zr ascorbate

Seventy-two percent of the theoretical value of the Zr citrate was measured after completing the dosage, and 42% of the zirconium concentration was determined after 24 h in the fermentation medium. This finding was supported by the examination of the Zr content of the yeast cells, too.

Examination of the Zr concentration of the yeast cells was in good agreement with the concentration of Zr in the fermentation media. The Zr ascorbate complex was better accumulated by yeast cells than the Zr citrate complex. Using the Zr complex the Zr content of the cells was 4300 μg Zr g^{-1} dry mass, while in the case of citrate complex it was 1600 μg Zr g^{-1} dry mass.

2.4. Change of amino acid content

The effect of the Zr-complexes on the amino acid content of the cell mass is presented in Figs 7 and 8. Data are not shown when the increase was below 10% compared to the control. Significant increase of concentration of Thr, Pro, Phe and Cys and decrease of Glu, Arg, Ser and Met were measured after addition of the Zr-ascorbic complex compared to medium containing ascorbic acid.

With the exception of Cys treatment with citric acid complex the amino acid concentration decreased compared to that of *S. cerevisiae* grown in the medium containing citric acid.

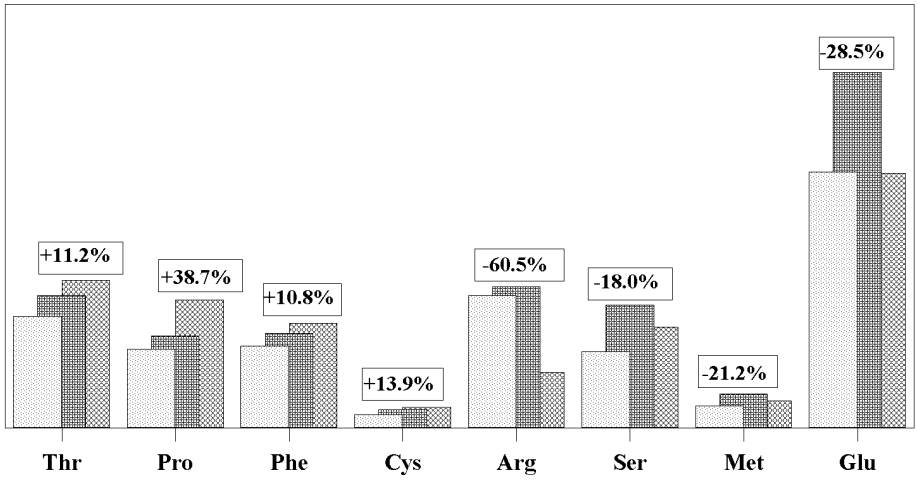

Fig. 7. Effect of the Zr ascorbate on the amino acid content of *Saccharomyces cerevisiae.*
Fermentation medium: ▭: Basic; ▦: basic medium + ascorbic acid; ▨: basic medium + Zr ascorbate

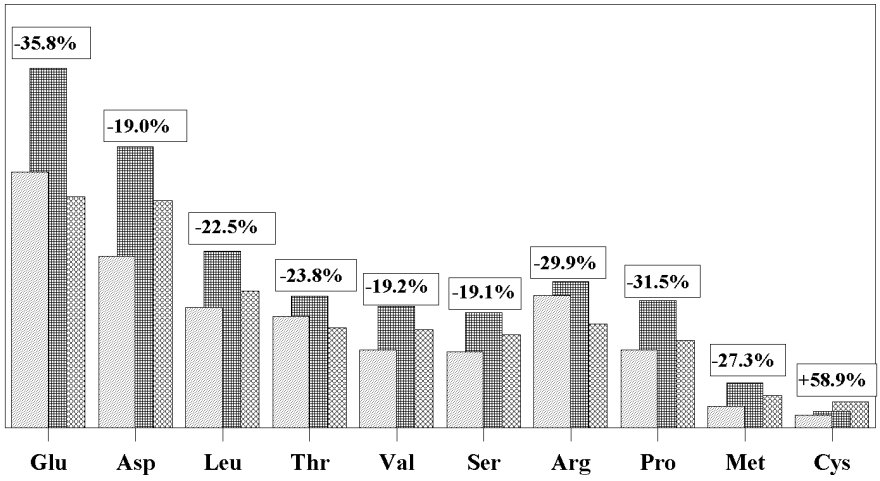

Fig. 8. Effect of the Zr citrate on the amino acid content of *Saccharomyces cerevisiae.*
Fermentation medium: ▭: Basic; ▦: basic medium + citric acid; ▨: basic medium + Zr ascorbate

Table 1. Total amino acid content of *Saccharomyces cerevisiae* (Y00891)

Medium	Amino acid concentration mg g^{-1}	Difference
Basic	394	
Basic medium + ascorbic acid	544	+38%
Basic medium + Zr ascorbate	490	–10%
Basic medium + citric acid	550	+40%
Basic medium + Zr citrate	456	–17%

The concentration of total amino acids is summarised in Table 1.

Several data in the literature prove that titanium (Ti), in the form of water-soluble chelate, can stimulate both plants and animals, which can be explained with the increase of enzyme activities. Ti increases the crop of different plants and affects the internal parameters beneficially. In animals Ti stimulates growth, improves feed conversion and it can have an immunomodulatory effect resulting in increased resistance to diseases (PAIS, 1983; ALCARAZ et al., 1994).

Since Zr is in the same subgroup as the Ti, and it has similar chemical properties it was supposed that Zr may have beneficial effect on eukaryotes, too. *S. cerevisiae* was chosen as a suitable model organism, and several similar investigations have already been carried out with this species.

Ascorbic acid and citric acid were used in production of complexes, and were chosen since they occur in the nature. In spite of the data of other workers (BLUMENTHAL, 1958) Zr formed a stabile complex with the ascorbic acid and its biological effect could be examined. The complex formation is a time reaction and considerable ligand excess was needed. That is why not only the biological effect of the complex but that of the excess ligand should be examined.

Both ascorbic acid and citric acid increased the OD of the fermentation media after 5–7 h compared to the basic medium, while Zr ascorbate and Zr citrate complexes slightly decreased the OD, showing inhibitory effect of Zr. This inhibitory effect was smaller in the case of Zr ascorbate and higher in the presence of Zr citrate. The cell mass was increased by both complexes as Zr ascorbate was accumulated from the fermentation medium by the yeast cells better and faster (Fig. 6). This phenomenon can be explained on one hand with the inhibitory effect of Zr citrate and on the other hand with the structure of Zr ascorbate, which has a more symmetrical structure than the Zr citrate, and the polarity of the ascorbate complex is lower and the membrane transport is easier.

The total amino acid content of the yeast cells was increased with the addition of ascorbic acid or citric acid to the fermentation media, but adding Zr ascorbate or Zr citrate decreased the positive effect of the ligands. The concentration of some amino acids was increased by 10–39% in the case of the ascorbate complex (Thr, Pro, Phe, Cys), while that of others decreased (18–60%). The Zr citrate decreased the amino acid concentration (19–32%), except the Cys. Further examinations are needed to understand how can Zr ascorbate increase the activity of certain enzymes and inhibit others.

The enzymes playing a role in amino acid synthesis are affected by Zr compounds but not to the same degree. It can be the reason of the difference in amino acid contents.

It is also conceivable that Zr links to other biological macromolecules, such as RNAs (similarly to titanium), influencing the process of protein biosynthesis. Further studies are necessary to ascertain how Zr is stored by the yeast.

A conspicuous result is the high Cys content decrease at the presence of Zr citrate in comparison to control yeast. A possible reason of this decrease is that high Zr citrate level may catalyse the breakdown of Cys.

References

ALCARAZ, C.F., CARVAJAL, M., FRUTOS, M., GIMÉNEZ, J.L., MARTÍNEZ-SÁNCHEZ, F. & PASTOR, J.J. (1994): The physiological role of titanium in *Capsicum annuum* L. plants. A review of the Spanish research during the 1898–1994 period. -in: PAIS, I. (Ed.) *Proceedings 6 th International Trace Element Symposium*. KÉE, Budapest, pp. 75–111.

BLUMENTHAL, W.B. (1958): *The chemical behaviour of zirconium*. D. van Nostrand Company, Inc, New York, pp. 139–150.

COUTURE, P., BLAISE, C., CLUIS, D. & BASTIEN, C. (1989): Zirconium toxicity assessment using bacteria, algae and fish assays. *Water, Air, and Soil Pollution*, *47*, 87–100.

ERDEY, L. (1956): *Bevezetés a kémiai analízisbe*. (Introduction to chemical analysis.) Tankönyvkiadó, Budapest, pp. 206–210.

HEGÓCZKI, J., JANZSÓ, B. & SUHAJDA, Á. (1995): Preparation of titanium enriched *Saccharomyces cerevisiae*. *Acta Alimentaria*, *24*, 181–190.

HEGÓCZKI, J., SUHAJDA, Á., JANZSÓ, B. & VERECZKEY, G. (1997): Preparation of chromium enriched yeasts. *Acta Alimentaria*, *26*, 345–358.

KÖRÖS, E. (1980): *Szervetlen biokémia*. (Inorganic biochemistry.) Gondolat, Budapest, pp. 16–57.

MERTZ, W. (1993): The history of the discovery of the essential trace elements. -in: ANKE, M., MEISSNER, D. & MILLS, C.F. (Eds) *Proceedings of the 8th International Symposium on Trace Elements in Man and Animals*. Verlag Media Touristic, Gersdorf, pp. 22–29.

PAIS, I. (1983): The biological importance of titanium. *J. Plant Nutr.*, *6*, 3–131.

PAIS, I. (1996): General overview on the development of trace element research. -in: HEMANTARANJAN, A. (Ed.) *Advancements in micronutrient research*. Scientific Publishers, Jodpour, India, pp. 1–18.

RÜTTNER, J.R., SPYCHER, M.A. & STOLKIN, I. (1987): Inorganic particulates in pneumoconiotic lungs of hard metal grinders. *Br. J. ind. Med.*, *44*, 657–660.

SAVVIN, S.B. (1961): Analytical use of Arsenazo III. *Talanta*, *8*, 673–685.

SCHARRER, K. (1955): *Biochemie der Spurenelemente*. Publisher Paul Parey, Berlin, pp. 229–231.

SIMON-SARKADI, L. & SZERZŐ, ZS. (1985): Ciszteinmeghatározási módszerek tanulmányozása. (Study of cysteine determination methods.) *Élelmiszerv. Közl.*, *31*, 203–207.

SNELL, F.D. (1978): *Photometric and fluorometric methods of analysis metals*. Vol. 2, Wiley, New York, pp. 1135–1181.

SUHAJDA, A., HEGÓCZKI, J., JANZSÓ, B., PAIS, I. & VERECZKEY, G. (2000): Preparation of selenium yeasts I. Preparation of selenium-enriched *Saccharomyces cerevisiae*. *J. Trace Elem. Med. Biol.*, *14*, 43–47.

TÖMÖSKÖZI, S., LÁSZTITY, R., NAGY, J., SIMON-SARKADI, L., FODOR, K. & SZABÓ, M. (1993): Nem konvencionális fehérjeforrások az emberi táplálkozásban. (Non-conventional protein resources in human nutrition.) *Élelm. Ipar*, *5*, 137–141.

ZHONG, W., PARKINSON, J.A., GUO, M. & SADLER, P.J. (2002): Unusual features for zirconium(IV) binding to human serum transferrin. *J. biol. inorg. Chem.*, *7*, 589–599.