

The Effect of Different Concentrations of Organic and Inorganic Zinc on The Growth And Zinc Content in Yeast (*Saccharomyces Cerevisiae*)

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Abstract

Introduction: In the aquaculture industry, yeasts like other microorganisms such as algae, play a major role. Yeasts can absorb minerals from their culture and is able to transfer nutritional materials to other organisms.

Materials and methods: In this study, the effect of three concentrations (2.5, 5 and 7.5 g L⁻¹) of organic (zinc-threonine) and inorganic (zinc-sulfate) zinc in culture media on the growth and enrichment of *Saccharomyces cerevisiae* was investigated. One group was also selected as the control treatment (each with 3 repetitions). Yeast culture was performed under standard conditions in YEPD medium and in 1-liter flasks. The amount of yeast added in the culture media was 10 g L⁻¹.

Results: Based on results, the most growth inhibitory of yeast was observed after adding 2.5 g L⁻¹ of zinc threonine, and with increase in zinc amount in culture media, the growth rate increased but still significantly lower than zinc sulfate and control treatments. In the zinc sulfate groups, the growth inhibitory of yeast was observed after adding 7.5 g L⁻¹ of zinc sulfate, but other treatments did not show significant difference with the control treatment. In this study, the highest zinc content (115.67 ± 4.65 mg g⁻¹) in yeast was observed in the treatment 7.5 g L⁻¹ of zinc sulfate, which was significantly higher than other treatments ($P < 0.05$). Consequently, zinc content of yeast in the zinc threonine group were lower than the zinc sulfate groups but in zinc threonine groups, the maximum zinc content (23.07 ± 1.14 mg g⁻¹) was observed in the treatment 5 g L⁻¹ of zinc threonine, which was significantly higher than other treatments of this group ($P < 0.05$).

Discussion and conclusion: The present study showed that yeast could be enriched with both forms of zinc, but zinc sulfate induces the least growth inhibitory in the yeast and also Zn content after enrichment was higher in zinc sulfate treatments than zinc-threonine treatments.

Key words: Organic and Inorganic Zinc, Baker Yeast (*Saccharomyces cerevisiae*), Growth and Enrichment

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Introduction

Development of the aquaculture industry is not possible without the use of live foods, especially those used in the early stages of larvae life, such as algae, yeasts, *Artemia*, rotifers and daphnia (1). Yeasts are mono-cellular fungi, without rashes and are seen in spherical and elliptic forms. Different types of yeasts, such as *Saccharomyces cerevisiae*, *Candida*, *Rhodotorula*, *Dipodascus capitatus*, and carotenoid yeast (*Phaffia rhodozyma*) are used to feed a variety of aquatic species, mass culture and enrichment the live food, such as rotifer and *Artemia* (2). Yeasts are the most important and mostly-used microorganisms in the food industry for production of yeast extracts (3). Typically, yeasts are used alive for the feeding of live food or used as an additive food in the aquaculture industry (4).

Saccharomyces cerevisiae due to the proper cell diameter (4-5 μ m), desirable nutritional value (a suitable source of proteins, vitamins, nucleotides and polysaccharides), cellular resistance, lack of leakage the organic soluble materials in the medium, easy preparation and affordable prices for aquaculture, especially live food, has been widely used in research. Also in certain physicochemical environments, yeasts are able to accumulate various minerals and form organic bonds at concentrations higher than normal (5). Baker's yeast is used as an interface to deliver nutrients to *Artemia* and other live foods. Adding minerals to baker's yeast makes it possible to produce live food containing sufficient amounts of minerals (6).

Zinc accumulation in yeast occurs in two steps. The first stage of bioavailability or *inactive adsorption* is related to accumulation on the cell wall

surface. The second stage is the active accumulation or *absorption stage*, which is associated with intracellular metabolism and intracellular penetration of ions by cell membrane and cellular circulation (7).

Although only a small amount of minerals is present in the body, zinc is known as an essential ingredient in aquatic nutrition and acts as a cofactor in many active enzyme systems. Therefore, the activity of aldolase, peptidases, and phosphatases that interfere with digestion is dependent on zinc (8). Zinc is a significant ingredient in larvae, because it is necessary for the growth, development and maintenance bone structure and its deficiency causes bones deformity. The zinc requirements of fish are 15 to 40 mg kg⁻¹ of zinc in diets (9). This amount is not available in live foods for larvae (10). Live foods such as zinc-enriched *Artemia* and rotifer can improve growth and prevent stress in nourished fish (11). Based on the need for minerals in live foods, yeast can be used as an interface for maximizing these materials. Zinc is also an essential element for the growth and metabolism of *Saccharomyces cerevisiae*. Zinc deficiency in the yolk formation stage prevents growth and causes the cells to stop in the G1 phase and in this case, Zn recruitments of yeast cannot be compensated by other metal ions. Zinc in metabolism plays a role in the production and operation of many enzymes, proteins, nucleic acids, gene expression and cellular immune development (12). Therefore, in the present study, the effect of different concentrations of organic (Zinc-threonine) and inorganic (ZnSO₄.7H₂O) zinc on the growth and zinc content of yeast (*Saccharomyces cerevisiae*) was investigated.

Materials and methods

Preparation of the materials:

Saccharomyces cerevisiae purchased from Iranian Biological Research Center. Yeast extract, Glucose, inorganic zinc ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and K_2HPO_4 were obtained from Jahan Kimia Company in Urmia. Threonine amino acid was purchased from Aryan Roshd Company.

Zinc-threonine preparation (organic form of zinc): Zinc-threonine was obtained by mixing the threonine amino acid with zinc sulfate. The solution of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (50.6 mg zinc sulfate in 6 ml of 50% ethanol) was added as a dropper to a threonine solution (5 ml ethanol 50%, final concentration of 0.016 mol.l^{-1}). Then, 100 μl of water was added to it, stirred continuously for an hour at room temperature, and finally at 8000 rpm the solution was centrifuged for 15 minutes (13).

Enrichment of Yeast: The yeast culture medium (YEPD) was prepared with yeast extract (2%), glucose (5%) and K_2HPO_4 (1%) and after adjusting the pH to 5.5, the culture medium was autoclaved at 121°C for 15 minutes (13). Organic and inorganic zinc forms were used to enrich yeast in YEPD medium. In summary, 10 g of yeast was grown in 1000 ml of YEPD culture medium at pH 8.5 and then incubated in an incubator shaker at 160 rpm at 27.4°C for 24 hours. After 24 hours of yeast incubation, two organic and inorganic zinc forms with 3 concentrations (2.5, 5 and 7.5 g L^{-1}) were added to the sterile culture medium of yeast and the incubation for another 24 hours continued with the same conditions. Then, the sterile medium was centrifuged with 3000 rpm for 15 minutes and washed twice with sterile physiological serum to remove the excess. The enriched yeast was then stored at -20°C (14).

Experimental design: In the present study, the effect of organic zinc threonine

($\text{C}_8\text{H}_{20}\text{N}_2\text{O}_8\text{Zn}$) and inorganic zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) on the growth and yeast enrichment in the 7 treatments including 1) control (yeast without adding zinc source), 2) yeast + 2.5 g L^{-1} zinc sulfate, 3) yeast + 5 g L^{-1} of zinc sulfate, 4) yeast + 7.5 g L^{-1} of zinc sulfate, 5) yeast + 2.5 g L^{-1} zinc threonine 6) yeast + 5 g L^{-1} zinc threonine and 7) yeast + 7.5 g L^{-1} zinc threonine on growth and enrichment of yeast were investigated.

Evaluation the yeast cells: After the preparation of the YEPD culture medium, the yeast was added. The number of yeast cells was immediately counted by hemocytometer lam. Also, 24 hours after adding zinc, the number of cells in the samples was counted (13).

Analysis procedure: 0.1 gram of Zn-enriched yeast was poured into a 200 ml flask and then 3 ml of concentrated nitric acid was added until the steam evolved (indicating the digestion of the sample), then nitric acid added to 10 ml. The amount of absorbed zinc in the yeast cell was measured by atomic absorption (15).

Statistical analyses: Data were analyzed by SPSS statistical software (version 21) using 2-way ANOVA. Levene's test were used for checking the homogeneity of variances ($P \leq 0.05$). Significance level between treatments was tested by Tukey's honestly (HSD) post-hoc test. Differences among means were considered at $P \leq 0.05$ and the data were shown as mean \pm SD.

Results

Determination of yeast growth in YEPD medium before adding zinc: The number of yeast cells at the first 24 hours after culture is shown in Figure 1. Based on t-test, the number of yeast cells increased 24 h after the culture compared to the beginning, and a significant difference was observed ($P < 0.05$).

Growth of yeast cells after adding zinc threonine and zinc sulfate: Results showed that the zinc source and zinc concentration significantly affected the yeast cell count (Table 1). Also there was a significant interaction between zinc source and zinc concentration on yeast cell count (Table 1).

The number of yeast cells 24 hours after adding zinc threonine and zinc sulfate were shown in Figure 2. In treated groups, the most growth inhibitory of yeast was observed in treatment 2.5 g L⁻¹ of zinc

threonine ($P < 0.05$). By increasing the concentration of zinc threonine, the amount of inhibition decreased. Also in this group, the highest number of yeast cell has been shown in the treatment of 5 g L⁻¹ of zinc sulfate and significant differences were observed among treatments ($P < 0.05$). In the treatment of 7.5 g L⁻¹ of zinc sulfate, the number of yeast cells decreased significantly compared to the control group ($P < 0.05$).

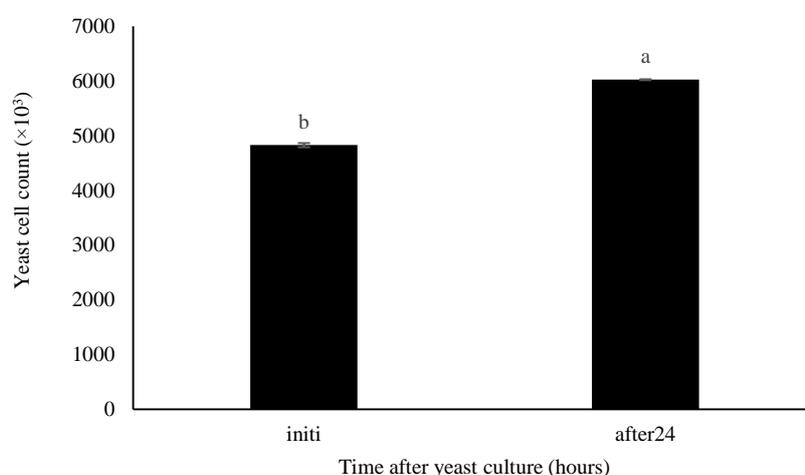


Figure 1- The number of yeast cells (mean \pm SD) for 24 hours before adding zinc

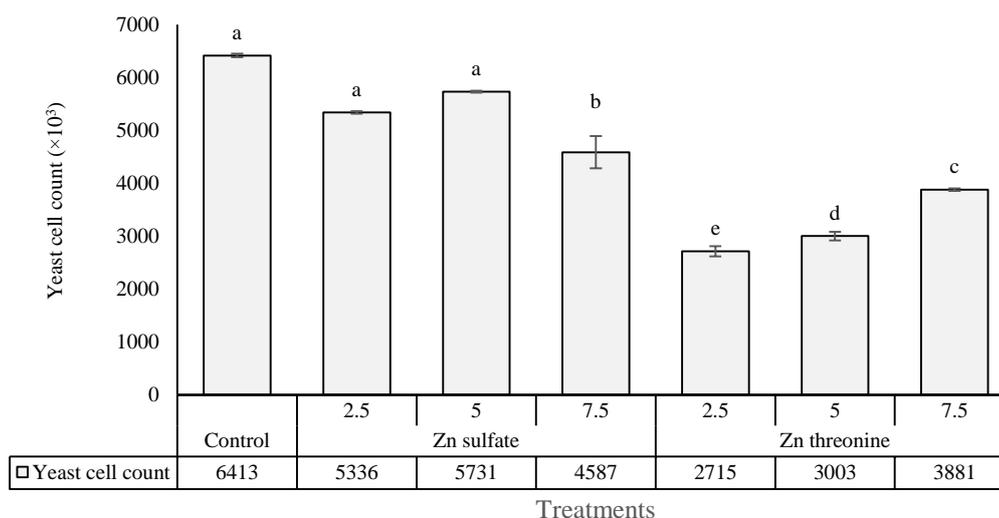


Figure 2- The effect of zinc source and zinc concentration on the yeast cell count, the number in horizontal axis indicates the amount of zinc sulfate and zinc threonine (g L⁻¹). Different letters have a significant difference ($P < 0.05$). The data were shown as mean \pm SD.

Table 1- Two-way ANOVA of the effect of different Zn source and concentration on Zn content and cell count in a growing population of yeast, *Saccharomyces cerevisiae*

Parameter	Source	SS	df	MS	F
Zn content in yeast	Zinc source	9782.459	1	9782.459	1960.756**
	Zinc concentration	11643.17	3	3881.056	777.903**
	Source * Conc	9718.195	3	3239.593398	649.292**
	Error	79.826	16	4.989	
Yeast cell count	Zinc source	1.37×10^{13}	1	1.37×10^{13}	986.078**
	Zinc concentration	2.22×10^{13}	3	7.40×10^{12}	530.933**
	Source * Conc	8.46×10^{12}	3	2.82×10^{12}	202.359**
	Error	2.23×10^{11}	16	1.39×10^{10}	

Explanation: ** $P < 0.001$

Evaluation of zinc content in enriched yeast: Based on Table 1, the zinc source and zinc concentration significantly affected the zinc content of yeast. Also, the results showed that there was a significant interaction between zinc source and zinc concentration on the zinc content of yeast (Table 1). Figure 3 shows the zinc content of yeast on the measured atomic absorption in different treatments. This amount in three concentrations significantly was higher in zinc sulfate compared to zinc

threonine groups ($P < 0.05$). The maximum Zn content ($115.67 \pm 4.65 \text{ mg g}^{-1}$) significantly was observed at high concentration of zinc sulfate ($P < 0.05$). Also with increasing the zinc sulfate, the amount of zinc content of yeast was increased (Figure 3). In Zn-threonine treatments, the maximum amount ($23.07 \pm 1.14 \text{ mg g}^{-1}$) of Zn content of yeast was obtained in 5 g L^{-1} of zinc threonine ($P < 0.05$).

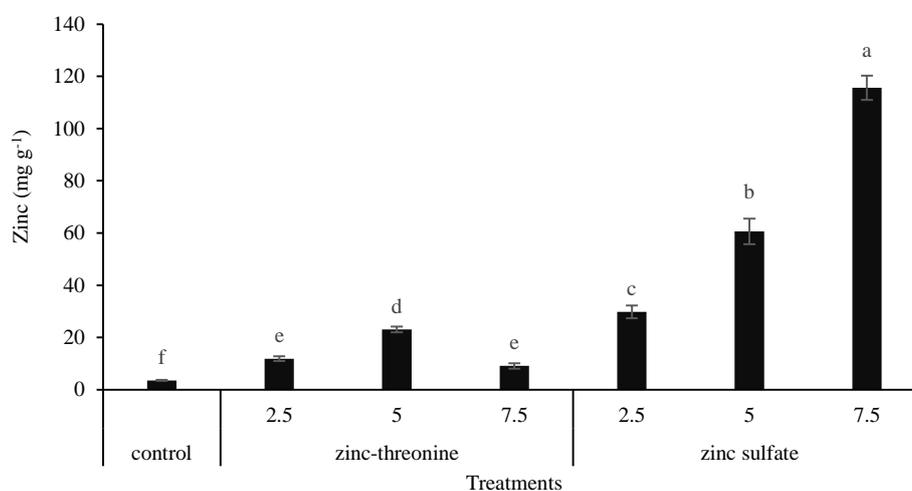


Figure 3- The effect of zinc source and zinc concentration on the zinc content of yeast, the number on horizontal axis indicate the amount of zinc sulfate and zinc threonine (g L^{-1}). Different letters have a significant difference ($P < 0.05$). The data were shown as mean \pm SD.

Discussion and conclusion

Yeast growth in YEPD culture medium was developed well for 24 hours (Figure 1) but the yeast cells count decreased by 24 hours after adding Zn-threonine in all three treatments, and only treatment 7.5 g L^{-1} when used the zinc sulfate (Figure 2). In

zinc sulfate treatments up to 5 g L^{-1} , the cell growth did not show significant difference with a control treatment. The effect of zinc on cell growth of yeast *S. cerevisiae* was investigated by other researchers, and it has been observed that adding zinc to the culture medium

inhibited the growth of yeast cell, which was probably due to a reduction in cell division (16). Based on other's results, different concentrations of sodium selenite had an inhibitory effect on cell growth of *S. cerevisiae* and a decrease in the biomass of yeast cell was also observed with increasing selenium concentration (17).

In this study, the maximum (23.07 ± 1.1 and 115.67 ± 4.65 mg g⁻¹ respectively in zinc threonine and zinc sulfate) content of zinc in yeast cell indicated that the zinc adsorption of yeast in the culture medium containing zinc sulfate was much more than zinc threonine. It was shown (18) that the highest enrichment (700 µg g⁻¹) with zinc in *S. cerevisiae* is carried out under anaerobic conditions. It was reported that zinc absorption/adsorption of *S. carcinoma* cells in beer factory waste was 1.7 mg g⁻¹ of dry matter (19). In another study, zinc sulfate was used as a zinc source in yeast enrichment and the zinc content in yeast reached to be 4.976 mg g⁻¹ dry cells (20).

It has been reported that zinc content in the yeast cell is highly dependent on zinc concentration in the culture medium (21, 16 and 22). The amount of absorbed zinc in the form of zinc nitrate and at a concentration 2 g L⁻¹ of the YEPD culture medium was 18.5 mg g⁻¹ of dry matter (21). Also it was found that with increasing concentrations of zinc sulfate in the culture medium (16) and addition the zinc sulfate in the culture medium up to 30 mg L⁻¹ (22), the zinc accumulation in yeast increased which is line with our study.

The results of the enrichment of yeast with other minerals indicated that the yeast cell has the ability to be enriched with them. The amount of absorbed metals by *S. cerevisiae* including copper and lead was 16 and 54.6 mg g⁻¹ of dry matter, respectively (23). Although it was reported (24) that the amount of adsorbed copper with dried *S. cerevisiae* was between 2.29-12.9 mg g⁻¹ of dry matter. The enrichment of *S. cerevisiae* with iron sulfate showed that cultured yeast cell contained 7.8 mg g⁻¹

¹ Fe of dry matter (20). Also, the enrichment of *Saccharomyces* with zinc, copper and manganese (18) concluded that under anaerobic conditions, the highest enrichment was achieved with zinc (700 µg g⁻¹ of dry matter) and in semi-aerobic conditions the highest enrichment observed with copper (1100 µg g⁻¹ of dry matter). The amount of adsorbed copper by *Saccharomyces* cells of beer factory waste was 1.8 mg g⁻¹ of dry matter (19).

In conclusion, *S. cerevisiae* had the ability to absorb/adsorb and to be enriched with zinc element by adding the zinc sulfate and zinc threonine, but the zinc content with zinc sulfate is greater than zinc threonine. Also, zinc sulfate had a less inhibitory effect on the growth of yeast cells than zinc threonine. Therefore, zinc sulfate up to 5 g L⁻¹ of culture medium of yeast is a better source for enrichment of *S. cerevisiae*.

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تأثیر غلظت‌های مختلف روی آلی و معدنی محیط کشت بر رشد و محتوای روی در مخمر نانوائی (*Saccharomyces cerevisiae*)

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چکیده

مقدمه: در صنعت آبی‌پروری مخمرها در کنار سایر میکروارگانیسم‌ها از جمله جلبک‌ها از اهمیت زیادی دارند. مخمرها می‌توانند مواد معدنی را از محیط کشت خود جذب کنند و به واسطه آن به عنوان یک انتقال دهنده برای رساندن مواد به موجودات تغذیه کننده در صنعت آبی‌پروری مطرح شوند.

مواد و روش‌ها: در پژوهش حاضر تاثیر ۳ غلظت (۲/۵، ۵ و ۷/۵ گرم در لیتر) روی آلی (روی ترئونین) و روی معدنی (روی سولفات) در محیط کشت بر رشد و میزان غنی‌سازی مخمر نانوائی بررسی شد. یک گروه به عنوان تیمار شاهد (هر کدام با ۳ تکرار) انتخاب شد. کشت مخمرها تحت شرایط استاندارد در محیط کشت YEPPD و در ارلن‌های یک لیتری انجام شد و مقدار مخمر اضافه شده در محیط‌های کشت ۱۰ گرم در هر لیتر بود.

نتایج: براساس نتایج بیشترین ممانعت رشد مخمرها بعد از اضافه کردن ۲/۵ گرم بر لیتر روی ترئونین مشاهده شد و با افزایش مقدار روی ترئونین در محیط کشت میزان رشد افزایش یافت. ولی همچنان نسبت به تیمارهای گروه روی سولفات و شاهد به طور معناداری پایین تر بود ($p < 0/05$). در گروه روی سولفات بیشترین میزان ممانعت رشد مخمرها بعد از اضافه کردن ۷/۵ گرم بر لیتر روی سولفات مشاهده شد. ولی سایر تیمارها با تیمار شاهد تفاوت معناداری را نشان ندادند ($p > 0/05$). در این مطالعه بیشترین مقدار روی اندازه‌گیری شده با جذب اتمی ($9/31 \pm 231/34$ میلی‌گرم بر گرم وزن خشک) در تیمار ۷/۵ گرم بر لیتر روی سولفات مشاهده شد که بطور معناداری بیشتر از سایر تیمارها بود ($p < 0/05$). به‌طور کلی مقدار روی در مخمر در گروه ترئونین کمتر از گروه روی سولفات بود اما در گروه روی ترئونین بیشترین مقدار روی اندازه‌گیری شده با جذب اتمی ($2/2 \pm 46/15$ میلی‌گرم بر گرم وزن خشک) در تیمار ۵ گرم بر لیتر مشاهده شد که به‌طور معناداری بیشتر از سایر تیمارهای این گروه بود ($p < 0/05$).

بحث و نتیجه‌گیری: مطالعه حاضر نشان داد مخمر با هر دو شکل روی معدنی و روی آلی غنی می‌شود ولی روی سولفات کمترین ممانعت رشد را در مخمر ایجاد می‌کند. همچنین، محتوای روی بعد از غنی‌سازی در تیمارهای روی سولفات بیشتر از تیمارهای روی ترئونین بود.

واژه‌های کلیدی: روی آلی، روی معدنی، مخمر نانوائی (*Saccharomyces cerevisiae*)، رشد و غنی‌سازی

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