Functional Food Based on Mineral-enriched Yeast Produced by High Efficiency Culture Technique

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너무나 고맙습니다. 이 작은 결실을 사랑하는 두 분께 바칩니다.

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Functional Food Based on Mineral-enriched Yeast Produced by High Efficiency Culture Technique

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Mineral is formed as a biological component and as nutrients. It is an essential component of the body that, when absent, makes life support impossible.

Generally, minerals are provided to animals and humans by plants and groundwater. But nowadays, minerals are not supplied in high enough amounts to

humans because of soil acidification. Nevertheless, 11 major elements (Fe, Zn, Cu, Mn, Co, I, Se, Mo, Cr, Mg, etc.) and 15 trace elements must be supplied to humans by any means, so artificial supplements have been developed to make up the shortage. Serious symptoms result from deficiencies in minerals, especially from selenium, iron, zinc, chromium, magnesium, and cobalt.

These minerals do not facilitate absorption in the body, so easily absorbed supplements have been needed. Mineral-enriched yeast is preferred more than other supplements, because its fermentation is recognized as safer than chemical materials and it looks like food. But in cultivating yeast, fermentation requires complicated facilities, skillful techniques, and high operation costs. Therefore, the productivity of mineral-enriched yeast has been very low and has not been able to overcome today's demand. But if the process of yeast cultivation can be improved, it is possible that productivity can be increased and become more economical.

The purpose of this study is to improve the mass production of mineral-enriched yeast (selenium, zinc, iron, chromium, cobalt, and magnesium) and increase the efficiency of the process without the possibility of contamination, waste water generation, and fermentation failure. The end result would be to improve the ability to produce functional foods and farm products containing high amounts of minerals coming from mineral-enriched yeast.

In this study, mineral-enriched yeast was produced by a new method using freeze-dried yeast. This method can replace the yeast culture step in the normal

fermentation process. Because the new method's process is simple, it is possible to reduce the costs of culture maintenance and the installation of the fermentation apparatus and eliminate the occurrence of wastewater. The study's experiments also confirmed that the theory of the new method was the same as that of normal culture. Mineral-enriched yeast is easy for people to eat and easy to feed to livestock. In this study, photosynthetic bacteria culture and feed additives were made of mineral-enriched yeast and functional foods with high mineral content were produced using the newly developed additives. These additives can control the amount of minerals fed to livestock and can also produce livestock products with high mineral content. When people eat these livestock products, they will not need to take medication-based mineral supplements.

Key words: Mineral, Selenium, Zinc, Iron, Chromium, Magnesium, Cobalt, Yeast, Photosynthetic bacteria, Functional food

Ⅰ. Introduction

Mineral is formed as a non-biological material, as a biological component, and as nutrients. Minerals are not simple constitutive components that support the three nutrients of protein, fat, and carbohydrate; they are essential components that make life impossible if they are missing from the body [1, 2].

Generally, minerals are provided to animals and humans by plants and groundwater. But nowadays, minerals are not supplied in high enough quantities to humans because repeated cultivation in the same fields has caused minerals to dwindle from soil acidification [3-5]. Nevertheless, 11 major elements (Fe, Zn, Cu, Mn, Co, I, Se, Mo, Cr, Mg, etc.) and 15 trace elements must be supplied to humans by any means [6, 7], so artificial supplements have been developed to make up the shortage [8]. Deficiencies of selenium, iron, zinc, chromium, magnesium, and cobalt especially have serious symptoms [9, 10].

Selenium (Se) is recognized as important in animal metabolism [11]. Selenium is a constituent of glutathione peroxidase (GSHpx) as an antioxidant [12]. Se occurs naturally in foods and the amount of Se contained in most feed and food plants varies according to the concentration and bioavailability of Se in the soil [13, 14]. The symptom of Se deficiency is severe, but an overload of Se is dangerous to animals as well [15].

Iron (Fe) is an essential mineral for humans and it affects various activities of life. Iron deficiency is a significant nutritional problem affecting women, children, and older people [16-18]. Iron is a component of hemoglobin; therefore, iron deficiency causes anemia, especially in women because of menstruation. Heme iron was used widely as an artificial iron supplement but it is not good for absorption and bioavailability for humans. Moreover, bovine spongiform encephalopathy was the cause of the prohibition of heme iron. Lactoferrin has better iron solubility in animal intestines for enhancing absorption, but it is expensive [19].

Zinc (Zn) exists in all areas of the human body and is a component of more than 300 enzymes [20]. Zinc deficiency seriously suppresses growth and immune system functions, as well as epidermal, gastrointestinal, central nervous, skeletal, and reproductive systems. Therefore, zinc should always be supplied in sufficient amounts [21, 22]. The Korean recommended daily allowance (RDA) for zinc is 15 mg/day for men and 12 mg/day for women. Sources of zinc are oysters, shellfish, fish, meat, eggs, seaweed, nuts and seeds, and more. Phytates interrupt zinc absorption [23].

Chromium (Cr) is an essential trace element, but it is easily mistaken for harmful minerals [24]. It is mainly absorbed in the small intestine; the absorption

rate of inorganic chromium (such as $CrCl_3$ and $Cr2O_3$) is very low (0.5-1.0%). Absorbed chromium is transported throughout the body by binding to transferrin and β-globulin in the blood. The distribution of the absorbed chromium within the tissue is influenced by chemical form and intake. It is widely distributed in relatively low concentrations in all body tissues: skin, fat, brain, muscles, spleen, kidneys, testicles, and so on. There should be 1-30 ppb of chromium in the blood. If blood glucose levels are increased or affected by disease, the concentration of chromium in the blood is reduced. Chromium is an essential component of glucose tolerance factor (GTF) and promotes interaction between insulin and insulin receptors on the surface of cells. It helps to enhance the function of insulin in the adsorption of glucose by the cell [25]. Cr promotes the hydrolysis of fat, which increases protein synthesis by providing energy and carbon sources. Cr helps to improve immunological responses and increases resistance to stress [26-28]. Absorbed chromium is mainly excreted through the kidneys in urine; the remainder is excreted by the hair, sweat, feces, and bile.

Magnesium (Mg) exists in about 20~28g in the human body and is divided into about 65% in the bone mineral contents, 34% in the muscles, and the rest in tissue and serum. It is the fourth most abundant mineral in the extracellular compartment and the second in intracellular. Magnesium plays an essential role in energy metabolism, the utilization of glucose, fatty acid metabolism, protein synthesis,

nucleic acid synthesis, and more than 300 hormone activities [29]. However, magnesium can interrupt calcium absorption.

The symptoms of magnesium deficiency are hypertension, congestive heart failure, alcoholism, eclampsia, and diabetes [30]. Magnesium deficiency was a common problem in hospital patients. The main excretion of Mg is performed through urine.

Cobalt is a component of vitamin B12. The symptoms of cobalt deficiency are similar to those of B12 deficiency: anorexia, weight loss, hyposthenia, and anemia.

Selenium, iron, zinc, magnesium, chromium, and cobalt do not facilitate absorption in the body, so easily absorbed supplements are needed. The traditional method of creating mineral-enriched yeast cultures was developed because the fermentation of mineral-enriched yeast cultures is safe from chemical materials and the cultures can be used with useful materials, such as various vitamins, trace elements, and protein in the yeast [31-39]. But this method requires complicated facilities, skillful techniques, and high operation costs.

After sterilizing the culture apparatus and liquid media, the yeast is cultured and incubated. When the most active growth of bacteria reaches a logarithmic

growth phase, the calculated nutrients and desired mineral salts are prepared and supplied together sterilely.

The remaining salt mineral on the exterior walls of the yeast is washed. The culture filtrate and wastewater must be treated specifically to prevent contamination, which costs a lot, so it becomes a major setback in producing mineral-enriched yeast. Along with this, it cannot be ignored that difficulties other than microbial contamination can occur during the yeast culture, and the production of the yeast can result in cells containing low efficient minerals.

Previous research on the production of mineral-enriched yeast has focused on increasing efficiency in the normal culture process. Most of the research has been on the optimization of the condition in which yeast is cultured to uptake as much mineral as possible. But the typical fermentation process is complicated and the cost of maintaining the apparatus is high, so the productivity of mineral-enriched yeast is very low and it cannot overcome the demand of today. Mass production is needed, but most cultivation facilities require a lot of investment, so if the operating rate of the fermenter is low, the risk of loss is very high.

If the cost of sterilization and the prevention of contamination can be reduced, the production costs of cultivation can be low. Therefore, when producing

mineral-enriched yeast, if the process of yeast cultivation can be improved, it is possible that productivity can be increased and become more economical.

Freeze-dried yeast can be made by vacuum drying after the quick freezing of yeast. There is no activity in dry yeast, but if water is supplied, the yeast's original characteristics begin. Dried yeast is normally used for baking, so it is possible to mass produce it at a low price commercially.

Using freeze-dried yeast makes it possible to get mass cell bodies before fermentation. So, keeping the principle and conditions of mineral yeast fermentation is a highly efficient and eco-friendly way to produce mineral yeast without the dangers of contamination and wastewater.

Mineral-enriched yeast is easy for people to eat and easy to feed to livestock [41-46]. Mineral-enriched yeast can be made into feed additives and the amount of minerals fed to livestock can be controlled. It is also possible to produce livestock products with a high mineral content [47-50]. When people eat these livestock products, they will not need to take medication-based mineral supplements.

The purpose of this study is to improve the mass production of mineral-enriched yeast (selenium, zinc, iron, chromium, cobalt, and magnesium) and

increase the efficiency of the process without the possibility of contamination, waste water generation, and fermentation failure. The end result would be to improve the ability to produce functional foods and farm products containing high amounts of minerals coming from mineral-enriched yeast.

Ⅱ. Materials and Methods

2.1 Strain and minerals

The freeze-dried yeast used in this study was Golden speed (DSM Bakery ingredients, UK). It is composed of 99% *Saccharomyces cerevisiae* and 1% rehydrate. The viability of the yeast was more than 1×10^9 colony forming units (CFU)/g and crude protein was 4~5%.

Sodium selenite (Na_2Seo_4), iron sulfate ($FeSO_4\cdot H_2O$), zinc sulfate ($ZnSO_4\cdot H_2O$), chromium chloride ($CrCl_3\cdot 6H_2O$), cobalt sulfate ($CoSO_4\cdot 7H_2O$), and magnesium sulfate ($MgSO_4\cdot 7H_2O$) were used as mineral sources.

2.2 Confirmation test that freeze-dried yeast absorbs minerals

An experiment was conducted to confirm that freeze-dried yeast absorbs minerals. The amounts of 50g of freeze-dried yeast with no treatment and another 50g of freeze-dried yeast were autoclaved at 121 ℃, 15 atm for 15 minutes, then prepared and put into 1,000 ml mass cylinders. Then 100 ml of water at 37 ℃ with 0.5 g of dissolved sodium selenite (Na_2SeO_4) was poured into each mass cylinder and mixed for a while to help start yeast life activation. After 2 hours, to confirm live yeast, the generation of CO_2 foam was checked in the mass cylinder

visually and an alcohol fermentation scent was checked through smell. As yeast absorbs more selenium, its color becomes redder, so it is possible to visually observe whether yeast has absorbed selenium or not. After 24 hours, the mixture was centrifuged to harvest a cake of yeast cells and the color of the cells was measured by the Roche yolk color fan (Roche, Switzerland but now this is produced by DSM, Netherland) to confirm that it had absorbed selenium.

2.3 Production of mineral-enriched yeast

2.3.1 Production of selenium-enriched yeast

1,000 g freeze-dried yeast was dissolved in 1,900 ml tap water at 37 ℃ and stirred at 400 rpm. 100 ml of 10% (w/v) sodium selenite (Na_2SeO_4) solution was added to the yeast mixture. The yeast produced carbon dioxide, so foam occurred in the mixture. To prevent overflow, 30% (v/v) edible antifoam agent was added to the yeast mixture. Mixing was carried out for 24 hours. After 24 hrs, a yeast cell cake was harvested by centrifuge and washed 3 times with clean water. The mineral-enriched yeast was dried with hot air and hydrolyzed with acid. The selenium concentration in the yeast was measured by an inductively coupled plasma mass spectrometer (ICP-MS).

2.3.2 Production of iron-enriched yeast

Iron-enriched yeast was produced by the same method for producing selenium-enriched yeast. 100 ml of 17% (w/v) iron sulfate monohydrate ($FeSO_4 \cdot H_2O$) solution was added to the yeast mixture. The iron concentration in the yeast was measured by the same analytical method.

2.3.3 Production of zinc-enriched yeast

Zinc-enriched yeast was produced by the same method for producing selenium-enriched yeast. 100 ml of 20% (w/v) zinc sulfate monohydrate ($ZnSO_4 \cdot H_2O$) solution was added to the yeast mixture. The zinc concentration in yeast was measured by same analytical method.

2.3.4 Production of chromium-enriched yeast

Chromium-enriched yeast was produced by the same method for producing selenium-enriched yeast. 100 ml of 15% (w/v) chromium sulfate hexahydrate

($CrCl_3 \cdot 6H_2O$) solution was added to the yeast mixture. The chromium concentration in yeast was measured by same analytical method.

2.3.5 Production of magnesium-enriched yeast

Magnesium-enriched yeast was produced by the same method for producing selenium-enriched yeast. 100ml of 30% (w/v) magnesium sulfate sevenhydrate ($MgSO_4 \cdot 7H_2O$) solution was added to yeast mixture. The magnesium concentration in yeast was measured by same analytical method.

2.3.6 Production of cobalt-enriched yeast

Cobalt-enriched yeast was produced by the same method for producing selenium-enriched yeast. 100ml of 15% (w/v) cobalt sulfate sevenhydrate ($CoSO_4 \cdot 7H_2O$) solution was added to yeast mixture. The cobalt concentration in yeast was measured by same analytical method.

2.4 Photosynthetic bacteria culture process

The strain and the medium for maintenance and culture used in this study were *Rhodobacter capsulatus* and Van Niel's yeast media composite 1.0 g K_2HPO_4, 0.5 g $MgSO_4$, 10.0 g yeast extract, and 20.0g agar per liter of distilled water.

The method of cultivation of the *Rhodobacter capsulatus* was as follows. The strain stored in the deep freezer was inoculated on Van Niel's agar plates and incubated at 35 ℃ for 48 hrs. To prepare the seed culture, the activated strain was inoculated with 100 ml of Van Niel's broth without agar in a 500 ml baffle flask and incubated at 35 ℃ with 500 LUX of light intensity in a rotary shaking incubator at 150 rpm for 48 hrs. For the main culture to contain selenium, 100 ml of seed culture was inoculated in sterilized 10 liters of Van Niel's broth and incubated at 35 ℃ with 500 LUX of light intensity, shaking the medium for 1 minute every 6 hours. Three days after inoculation, the culture broth's color was observed as having changed to green.

2.5 lysis of selenium-enriched yeast cell walls

A 5N hydrochloric acid solution was added to the selenium-enriched yeast culture, which finished the process of production before drying. The ratio was 1 ㎖ 5N hydrochloric acid solution per 1 g freeze-dried yeast. After adjusting to pH 7.0 with 5N sodium hydroxide, the culture was dried with hot air and the

morphology of the yeast was observed through a scanning electron microscope to confirm cell wall lysis.

2.6 Production of photosynthetic bacteria containing organic selenium

1.6 kg freeze-dried yeast and 109.1 g Na_2SeO_4 was used to make a selenium-enriched yeast culture containing 30,000 ppm (mg/kg) selenium. The yeast culture was degraded and sterilized in a wet state and added aseptically to the main culture of the photosynthetic bacteria 5 days after inoculation.

This culture was incubated for more than 3 days and 10 L photosynthetic bacteria culture containing selenium was produced. The selenium concentration in the cell and culture filtrate was measured by ICP-MS.

2.7 Production of functional agricultural food containing high mineral content

2.7.1 Selenium rice

Rice containing more than normal selenium was produced by spraying the rice with a photosynthetic bacteria culture of the selenium-enriched yeast. A

photosynthetic bacteria culture containing 5,000 ppm (mg/kg) selenium was diluted 2:1 with water and 5 liters were sprayed on a 50 ㎡ rice field 3 days before harvest. The harvested rice was threshed and separated into rough rice and brown rice. The selenium content of the rough rice and the brown rice was measured by ICP-MS.

2.7.2 Selenium pears

A photosynthetic bacteria culture containing 5,000 ppm (mg/kg) selenium was diluted 2:1 with water and 5 liters were sprayed on 5 pear trees before harvest. After 10 days, the pears were harvested and dried. The selenium content of the dried pear was measured by ICP-MS.

2.7.3 Selenium persimmons

A photosynthetic bacteria culture containing 5,000 ppm (mg/kg) selenium was diluted 2:1 with water and 5 liters were sprayed on 5 persimmon trees before harvest. After 20 days, the persimmons were harvested and dried. The selenium content of the dried persimmon was measured by ICP-MS.

2.7.4 Selenium leeks

A photosynthetic bacteria culture containing 5,000 ppm (mg/kg) selenium was diluted 2:1 with water and 5 liters were sprayed on a 50 ㎡ field that grew leeks about 10-20 ㎝ length. After 20 days, leeks were harvested and dried. The selenium contents of dried leek was measured by ICP-MS

2.7.5 Selenium cabbage

A photosynthetic bacteria culture containing 5,000ppm (mg/kg) selenium was diluted 2:1 ratio with water and 5 liters were sprayed on a 50 ㎡ cabbage filed. After 20 days, cabbage was harvested and dried. The selenium contents of dried-cabbage was measured by ICP-MS

2.8 Production of new feed additives with organic minerals

New feed additives with high concentrations of minerals were developed using selenium-, iron-, zinc-, chromium-, and magnesium-enriched yeast. The new feed additives were created to produce functional livestock products.

2.8.1 New feed additive containing organic selenium

22.7g Na_2SeO_4 was mixed with 1.0 kg freeze-dried yeast to make a selenium-enriched yeast culture containing 10,000 ppm (mg/kg) selenium. After cultivation, the yeast culture in a wet state was added to 99 kg rice bran and mixed together. The mixture was dried at 50 ℃ for 20 hours and then the concentration of the selenium in the produced 100 kg feed additive was measured by ICP-MS.

2.8.2 New feed additive containing organic iron

5.0 kg $FeSO_4 \cdot H_2O$ was mixed with 20.0 kg freeze-dried yeast to make an iron-enriched yeast culture containing 50,000 ppm (mg/kg) iron. After cultivation, the yeast culture in a wet state was added to 80 kg rice bran and mixed together. The

concentration of the iron in the 100 kg feed additive produced by the same method was measured by ICP-MS.

2.8.3 New feed additive containing organic zinc

680.0g $ZnSO_4 \cdot H_2O$ was mixed with 5.0 kg freeze-dried yeast to make a zinc-enriched yeast culture containing 50,000 ppm (mg/kg) zinc. After cultivation, the yeast culture in a wet state was added to 95 kg rice bran and mixed together. The concentration of the zinc in the 100 kg feed additive produced by the same method was measured by ICP-MS.

2.8.4 New feed additive containing organic chromium

150.0 g $Crcl_3 \cdot 6H_2O$ was mixed with 1.0 kg freeze-dried yeast to make a chromium-enriched yeast culture containing 30,000 ppm (mg/kg) chromium. After cultivation, the yeast culture in a wet state was added to 99 kg rice bran

and mixed together. The concentration of the chromium in the 100 kg feed additive produced by the same method was measured by ICP-MS.

2.8.5 New feed additive containing organic magnesium

5.0 kg $MgSO_4 \cdot 7H_2O$ was mixed with 20.0 kg freeze-dried yeast to make a magnesium-enriched yeast culture containing 25,000 ppm (mg/kg) magnesium. After cultivation, the yeast culture in a wet state was added to 80 kg rice bran and mixed together. The concentration of the magnesium in the 100 kg feed additive produced by the same method was measured by ICP-MS.

2.9 Production of functional livestock food containing high content mineral

2.9.1 Selenium chicken

20,000 2-week-old Ross broiler chickens were fed 0.2 ppm selenium for the production of chicken meat containing selenium. During the experiment period, the broiler chicks were fed a diet of Mok-woo-chon feed for 34 days. After

breeding, the concentrations of the selenium in the parts of the chickens (legs, wings, breasts, and skin) were measured by ICP-MS.

2.9.2 Selenium eggs

Nine 77-week-old laying hens were divided into 3 groups for 30 days to produce eggs containing selenium. Feed mixed with 0.2% feed additive containing selenium was fed to the chickens for 30 days. The final concentration of selenium in feed was 0.2 ppm and it was from Se-enriched yeast. The eggs produced on the 30th day were separated by egg yolk and egg white. These were dried in a freeze vacuum dryer and the selenium content of each was measured by ICP-MS.

2.9.3 Magnesium eggs

Eggs containing magnesium were produced by the same method as the production of selenium eggs. The final concentration of magnesium in feed was 10 ppm and it was from Mg-enriched yeast. The magnesium content in the dried egg yolk and egg white was measured by ICP-MS.

2.9.4 Iron pork meat

A total of six pigs post weaning were fed a diet of feed with a 0.1% feed additive containing iron. The final concentration of iron in feed was 10 ppm and it was from Fe-enriched yeast. The loins and hind legs were dried and the iron content of each was measured by ICP-MS.

Ⅲ. Results

3.1 Confirmation test that freeze-dried yeast absorbs minerals

The results of the confirmation test that freeze-dried yeast absorbs minerals are below. Generally, during the activation of freeze-dried yeast in water, yeast produces CO_2 gas and alcohol. In this study, water in which selenium was dissolved was poured into a 1,000 ml mass cylinder that included freeze-dried yeast. After two hours, the normal freeze-dried yeast produced 200 ml of CO_2 gas foam and alcohol. But the sterilized freeze-dried yeast did not produce CO_2 gas foam and no alcohol fermentation scent was detected. After 24 hours, both yeasts were harvested by centrifuge and washed with clean water and then the yeast cake color was compared (Table 1). The color of the normal freeze-dried yeast cake was number 15 red of the Roche yolk color fan (Figure 1) but the sterilized yeast was number 1. The color of the sterilized yeast did not change because it did not uptake any selenium due to no life activity. In conclusion, the freeze-dried yeast has life activity and the yeast uptake of selenium into a body not by the surface, which is the same way compared to the previous normal fermentation process.

Table 1. The results of the freeze-dried yeast availability test

Freeze-dried yeast	Sterilized	Normal
CO_2 generation (ml)	0	200
Odor	None	Alcohol scent
Color of yeast	Pale yellow No [1] 1. color fan	Red No [1] 15. Color fan

[1] No means the number of blades of the fan that can be evaluate the red grades of egg yolk color.

Figure 1. Yolk color fan (Roche. Switzerland)

Yolk color fan is produced by DSM, Netherland now. This fan is an essential tool for helping decide the red grade of color.

3.2 Results of production of mineral-enriched yeast

3.2.1 Se, Fe, Zn, Cr, Mg and Co content in yeast

The content of each mineral in the yeast produced by the new technique was measured in a high concentration the same as in the previous normal fermentation process. The purpose of the new technique is to increase production efficiency and lower production costs.

The analysis results are different from the calculated value but similar to the desired concentration (Figure 2). Therefore, the possibility of controlling the concentration of mineral content in yeast has been confirmed.

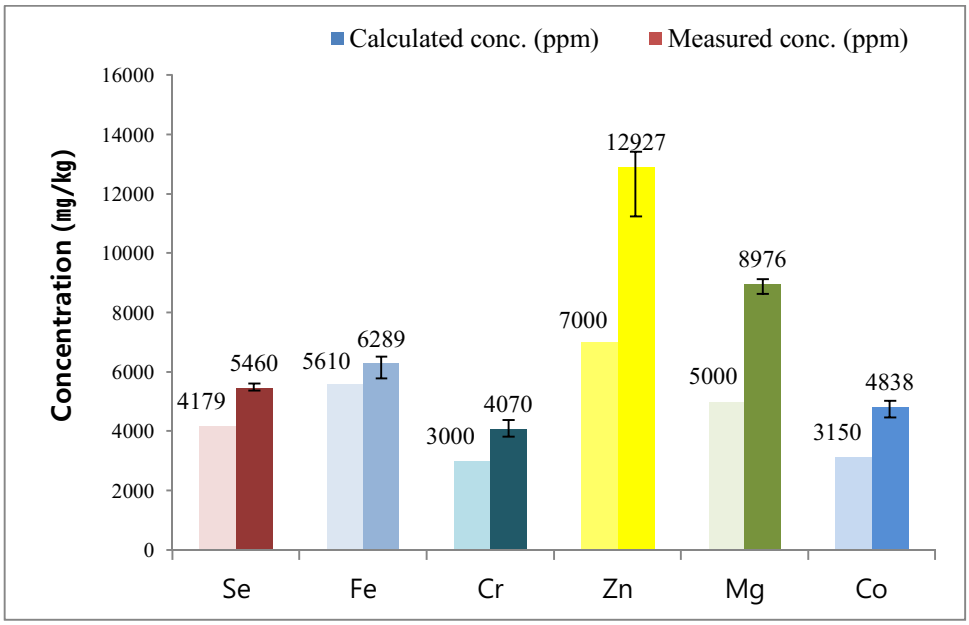


Figure 2. Individual results of Se, Fe, Zn, Cr, Mg and Co content in yeast

■ Calculated means theoretical value for controlling the concentration of mineral content in yeast.

■ Measured means analytical result of the concentration of mineral content in yeast.

Vertical bars represent the standard deviation of each data point(n = 3)

3.2.2 No generation of culture filtrate wastewater

In normal fermentation, 5,000 ml water is needed to obtain 500 g of dried yeast. But this new technique only used 900 ml of water, which was only 18% compared to normal cultures. In this study, water was used at 1.8 times of the freeze-dried yeast weight. The viscosity of the mixture was low and stirring was possible even with a further reduction in the amount of water. High viscosity cultures can be dried with a fluid bed dryer and low viscosity cultures can be dried using a spray drier of a disc drier. The new technique does not require a centrifugation process. Therefore, culture filtrate wastewater is not generated.

3.2.3 The elimination of the washing process

The freeze-dried yeast and minerals were mixed for 24 hours. To compare the mineral content, one was dried without washing and the other was washed 3 times and dried. The mineral content of the yeast without washing was measured as 90% of the initial dose and the washed yeast was measured as 73%. So, in producing mineral-enriched yeast using freeze-dried yeast, 30% of the yeast weight added at the beginning was able to absorb residual minerals. Theoretically, all the minerals were absorbed and the washing process could be eliminated.

Table 2. Absorption rate of Cr comparing with and without washing

	Calculated [1]	Measured [2]	
		Without washing	Washing
Cr content in yeast (㎎/㎏)	1,000	894	733
Absorption rate (%)	100	89	73
Cr content in yeast (㎎/㎏)	2,000	1660	1460
Absorption rate (%)	100	83	73

[1] Calculated means theoretical value for controlling the concentration of mineral content in yeast.

[2] Measured means analytical result of the concentration of mineral content in yeast.

$$\text{Absorption rate (\%)} = \frac{\text{Measured analytical result of Cr concentration in yeast}}{\text{Calculated theoretical Cr concentration in yeast}} \times 100$$

3.2.4 Comparison of the efficiency of the new method and the normal culture process

Theoretically, the volume of the fermenter requires twice the volume of the operation. For example, to obtain a dry weight of 2,000 kg, the volume of the operation must be 20,000 L and the volume of the fermenter should be over 30,000 L in a batch culture. But new technique was needed at 1.8 times water of the freeze-dried yeast weight, so it requires just 5,000 L mixing tank. Because the cost of apparatus installation was down, so efficiency was high up to 83% compared to normal cultures (Table 3).

The cost of sterilizing the medium and the cost of maintenance of optimum culture state are heeded oil and electric energy high. New technique was needs electric power for only mixing. New technique did not need the process of harvest.

The cost of treatment of the wastewater is high. After fermentation, the cleaning and the maintenance of the fermentation apparatus are needed and it is required cost too. Moreover, the fermenter is made of expensive parts because it has to be precise and a building is needed in which it can be installed. Thus, normal fermentation procedure costs a lot of money more than new developed method.

Table 3. Comparison of the efficiency of the new method and the normal culture process

	Normal fermentation	New method	Efficiency (%)
Apparatus	30,000 L fermenter	5,000 L mixing tank	Up 83
Energy consumption	Sterilization, culture maintenance, Cleaning	Only mixing	Up 90
Harvesting	Centrifuge	No	Up 100
Wastewater	20,000 L	0 L	Up 100
Washing water	20,000 L	0 L	Up 100
Dryer	Need	Need	Up 0
Contamination	Possible	Never	Up 100

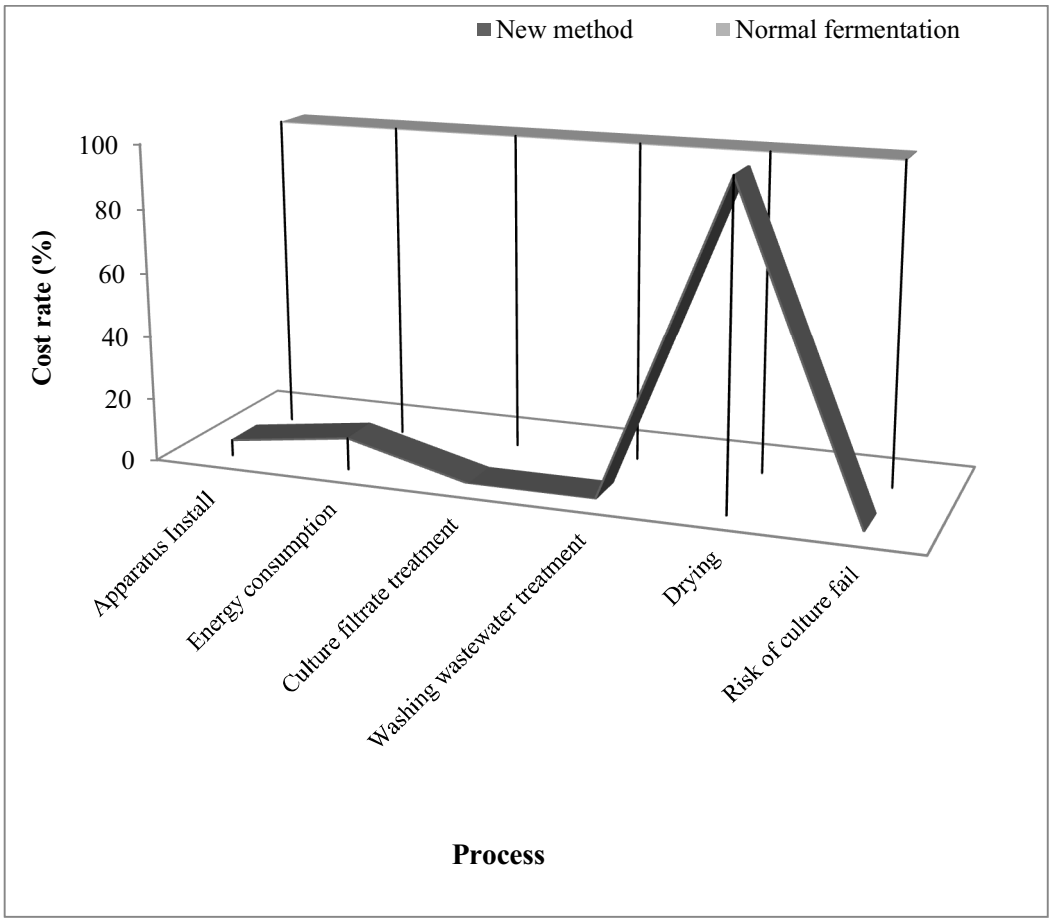
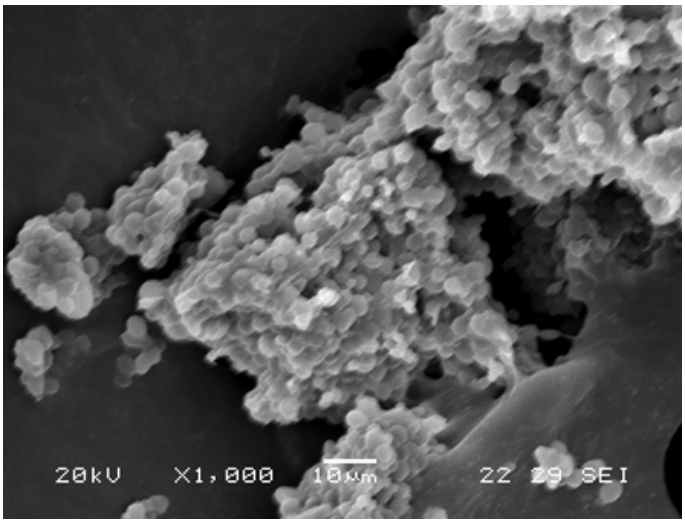


Figure 3. Comparison of the cost rate of the new method and the normal culture process

3.3 Result of the lysis of selenium-enriched yeast cell walls

The morphology of the selenium-enriched yeast cells before the 5N hydrochloric acid treatment was observed as a shell type through the scanning electron microscope. But the degradation of the yeast cell walls could be seen after treating it with HCl solution (Figure 4).

A



B

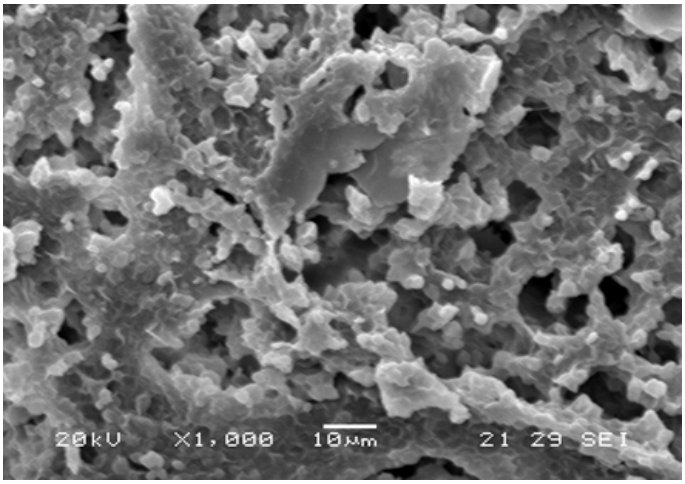


Figure 4. The photograph of yeast cell shape by scanning electron microscope.

A: The morphology of drying yeast powder before 5N hydrochloric acid treatment.

B : The morphology of drying yeast powder after 5N hydrochloric acid treatment.

3.4 Production of photosynthetic bacteria containing organic selenium

After the photosynthetic bacteria (PSB) containing organic selenium was cultured, the selenium content was measured. The culture broth was centrifuged to separate the PSB cell body and the culture filtrates. The content of selenium in the dried PSB cell powder was 70,511 ppm and in the culture filtrates was 1,312 ppm.

Selenium-enriched yeast with degraded cell walls was added in the middle of PSB cultivation. Both the PSB cells that absorbed selenium and the selenium bound to proteins in the yeast cells were settled together by centrifugation and they were measured together. This is the reason for the high content of selenium in the dried PSB powder.

The remaining selenium in the culture filtrate was combined with organic matter, so it can be used to produce functional food.

Table 4. Selenium content in photosynthetic bacteria cell powder and culture filtrate

PSB containing Se	Morphology	Se content (ppm)
Culture filtrate	Liquid	1312
Cell	Dried cell powder	70511

PSB : Photosynthetic bacteria

Se : Selenium

Liquid : The culture broth of photosynthetic bacteria without cell bodies.

Dried cell powder : The precipitation of photosynthetic bacteria cell with selenium-enriched yeast cell debris together.

3.5 Production of new feed additives with organic minerals

Selenium, iron, zinc, chromium, and magnesium-enriched yeast were used to make feed additives for the production of functional livestock products. When iron and magnesium are eaten together, they interfere with absorption in the small intestine, so they had to be made separately. After the cultivation, the mineral-enriched yeast cultures in a wet state before the drying process were mixed with rice bran. Therefore, no additional process was needed compared with making normal feed additives. The mineral content of the feed additives made of mineral-enriched yeast was measured as Se 100 ppm, Fe 10,000 ppm, Zn 5,000 ppm, Cr 300 ppm, and Mg 5,000 ppm.

Table 5. Results of the mineral content in the feed additives using Se-, Fe-, Zn-, Cr- and Mg-enriched yeast

	Se	Fe	Zn	Cr	Mg
Calculated (ppm) [1]	100	10000	5000	300	5000
Measured (ppm) [2]	109	9625	5422	295	5320
Rate (%)[3]	109	96	108	98	106

[1] Calculated means theoretical value for controlling the concentration of mineral content in yeast.

[2] Measured means analytical result of the concentration of mineral content in yeast.

[3] Rate (%) = $\frac{\text{Measured mineral concentration in mineral-enriched yeast}}{\text{Calculated mineral concentration in mineral-enriched yeast}} \times 100$

3.6 Production of functional agricultural and livestock food containing high mineral content

Functional agricultural foods were produced using selenium-enriched yeast, iron-enriched yeast, and magnesium-enriched yeast, which were prepared with the new technique using freeze-dried yeast.

Photosynthetic bacteria cultured with selenium-enriched yeast was used to produce agricultural products such as rice, pears, persimmons, leeks, and cabbage with increased selenium content (Figure 5, 6, 7, 8 and 9). The feed additive made from selenium-enriched yeast was also used to produce selenium-rich eggs (Figure 10) and chicken (Figure 11). The selenium content in the functional food can be controlled by adjusting the amount of selenium-enriched yeast. The recommended daily dose of selenium is 50-200 μg.

The feed additives prepared with iron-enriched yeast were used to produce pork with increased iron content (Figure 12). The recommended daily dose of iron is 50 mg for men and 14 mg for women.

An egg with increased magnesium content was produced using a feed additive prepared with magnesium-enriched yeast (Figure 13). The recommended daily dose of magnesium is 350 mg for men and 280 mg for women.

Milk and eggs with high chromium and zinc content and a red pepper with high selenium content were also produced (data not shown).

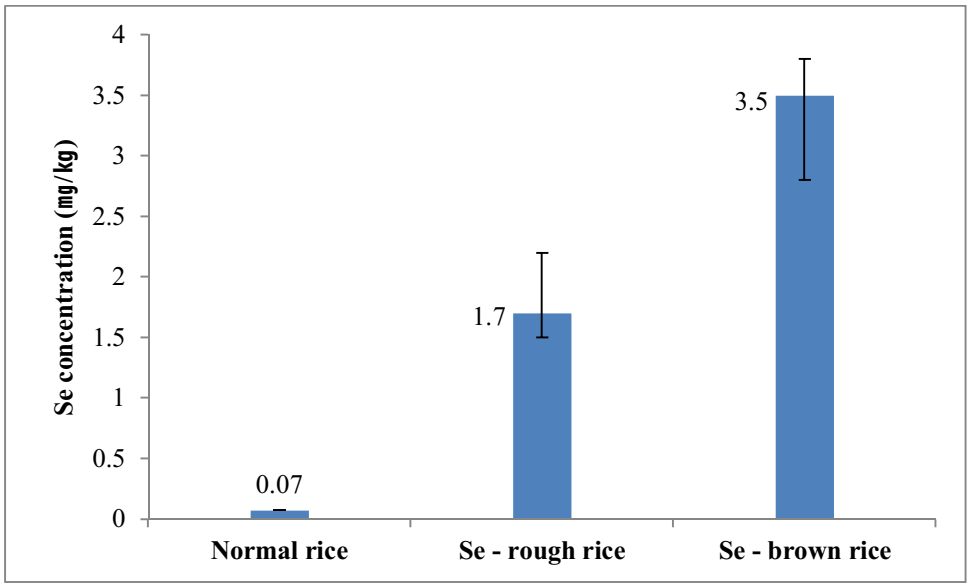


Figure 5. The concentration of selenium in rough rice and brown rice

Rough rice and brown rice was dried by hot air, so the concentration of selenium in a same rice before drying was lower than these values.

The moisture content of rice was 40%.

Vertical bars represent the standard deviation of each data point(n = 3)

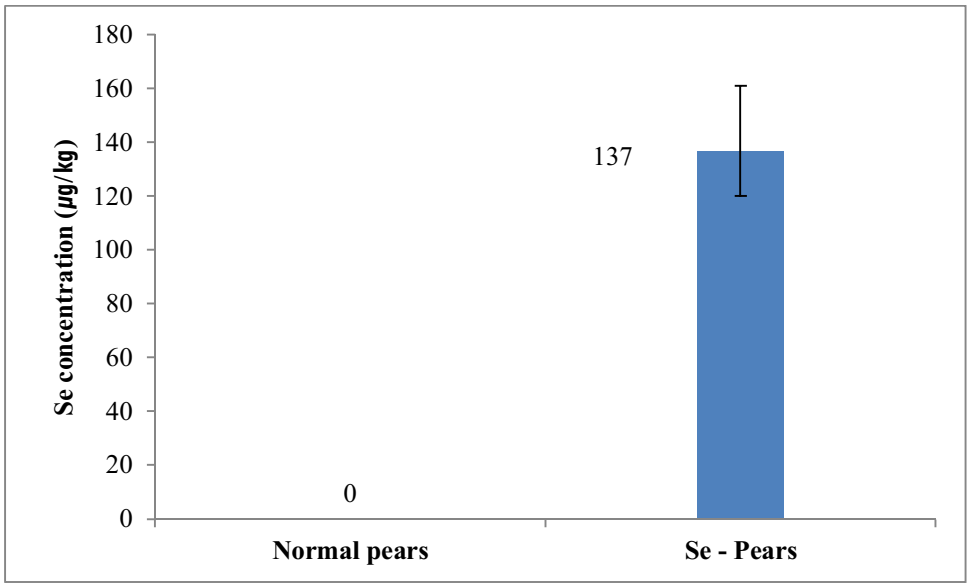


Figure 6. The concentration of selenium in pears

Se - pears were dried using vacuum dryer after quick freezing, so the concentration of selenium in a same pear before drying was lower than these values.

The moisture content of pears were 86%.

Vertical bars represent the standard deviation of each data point(n = 3)

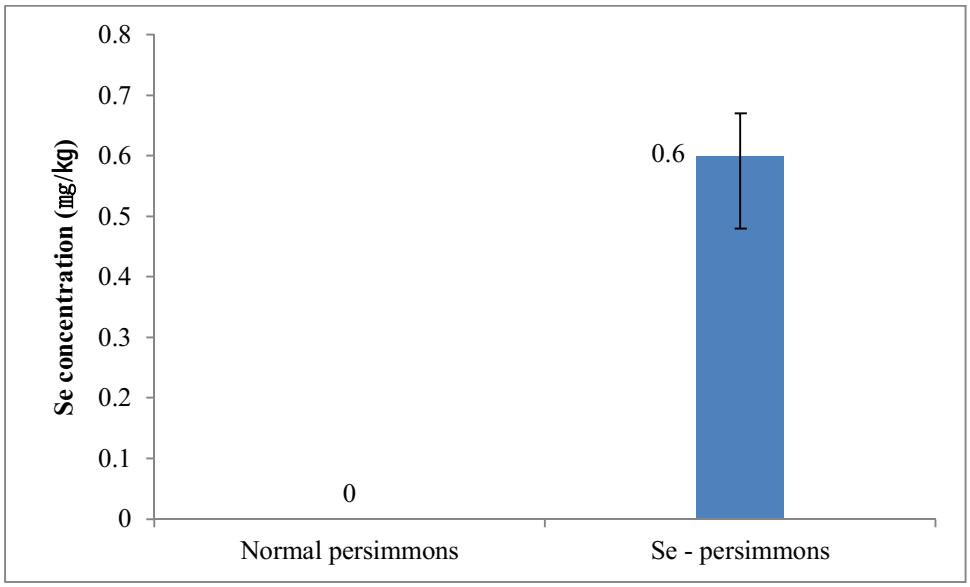


Figure 7. The concentration of selenium in persimmons

Se - persimmons were dried using vacuum dryer after quick freezing, so the concentration of selenium in a same persimmon before drying was lower than these values.

The moisture content of persimmons were 83%.

Vertical bars represent the standard deviation of each data point(n = 3)

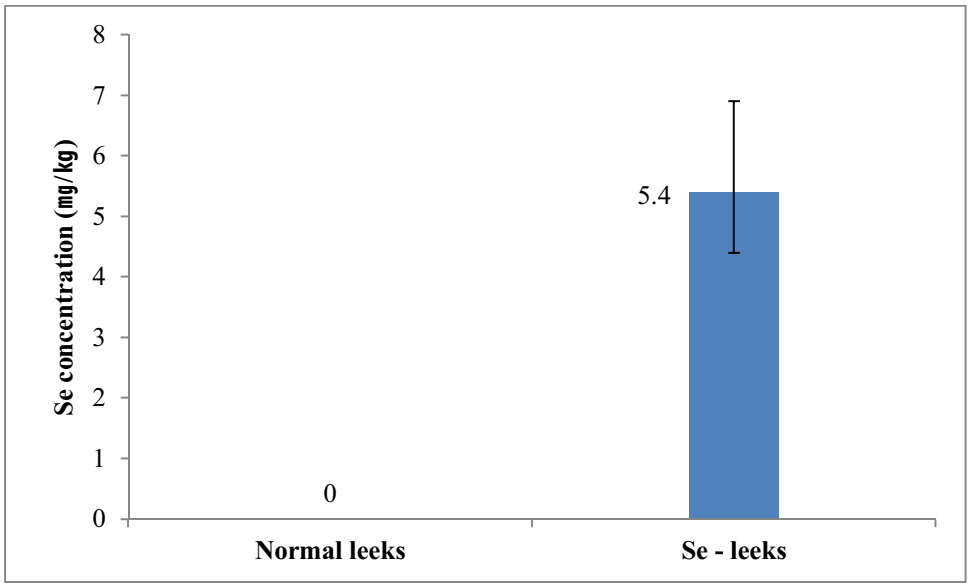


Figure 8. The concentration of selenium in leeks

Se - leeks were dried using vacuum dryer after quick freezing, so the concentration of selenium in a same leek before drying was lower than these values.

The moisture content of leeks were 60%.

Vertical bars represent the standard deviation of each data point(n = 3)

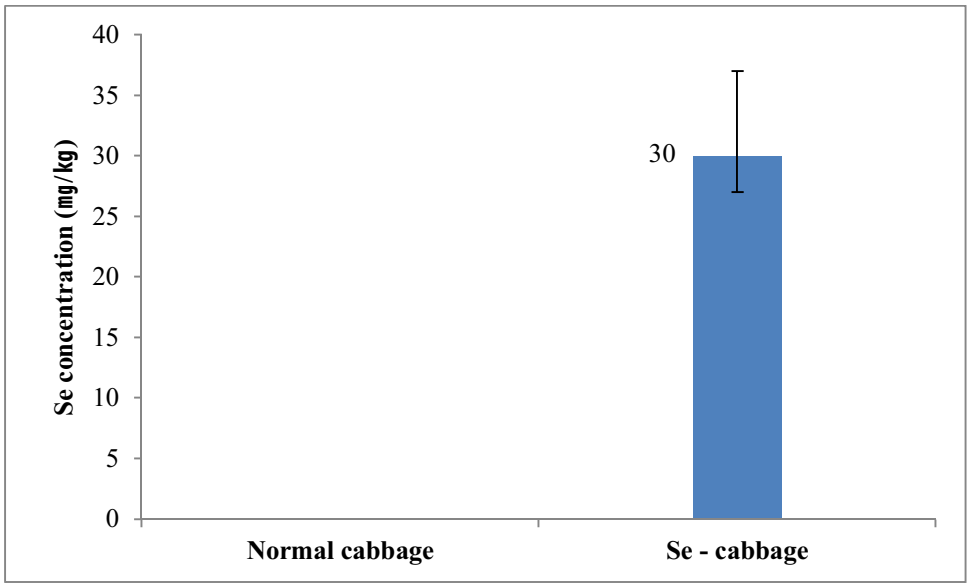


Figure 9. The concentration of selenium in cabbage

Se - cabbage was dried using vacuum dryer after quick freezing, so the concentration of selenium in a same cabbage before drying was lower than these values.

The moisture content of cabbage was 70%.

Vertical bars represent the standard deviation of each data point(n = 3)

The amount of selenium-enriched photosynthetic bacteria culture broth sprayed on growing pears, persimmons, rice, leeks, and cabbage was the same volume. Therefore, the pears and persimmons, which are larger than leeks, rice, and cabbage, have lower selenium content. Moreover, the pears were covered with paper bags to protect the fruit against harmful birds.

The content of selenium, iron, and magnesium can be controlled so as not to be too much in the crops.

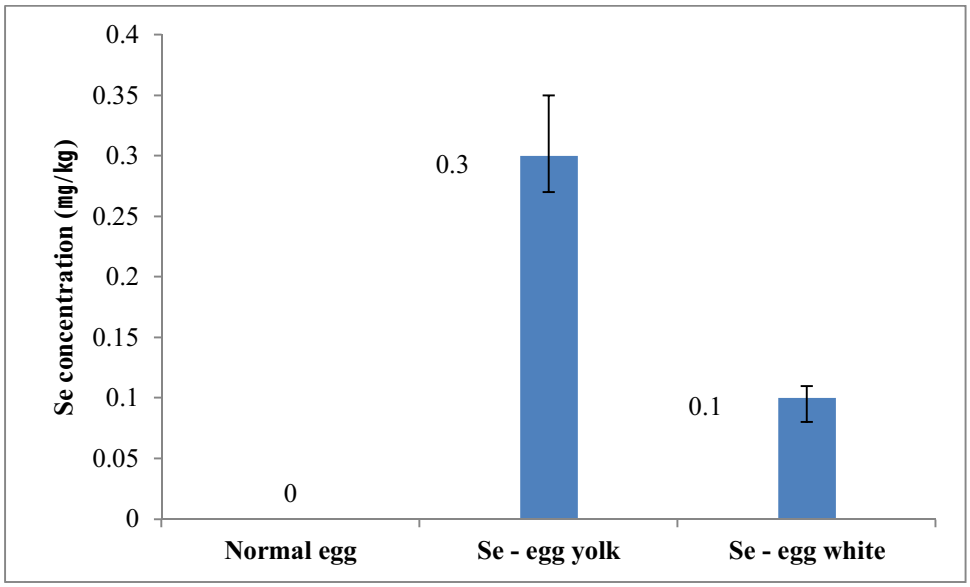


Figure 10. The concentration of selenium in the egg yolk and egg white from the same egg

Se - egg was dried using vacuum dryer after quick freezing, so the concentration of selenium in a same egg before drying was lower than these values.

The moisture content of egg was 75%.

Vertical bars represent the standard deviation of each data point(n = 3)

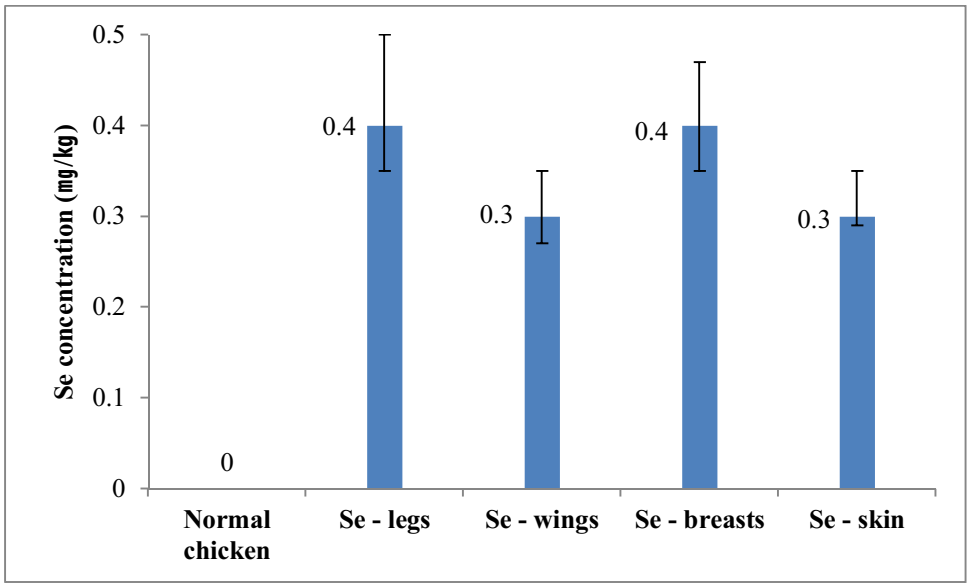


Figure 11. The concentration of selenium in the legs, wings, breasts and skin of a chicken

Se - chicken was dried using vacuum dryer after quick freezing, so the concentration of selenium in a same chicken before drying was lower than these values.

The moisture content of chicken was 66%.

Vertical bars represent the standard deviation of each data point(n = 3)

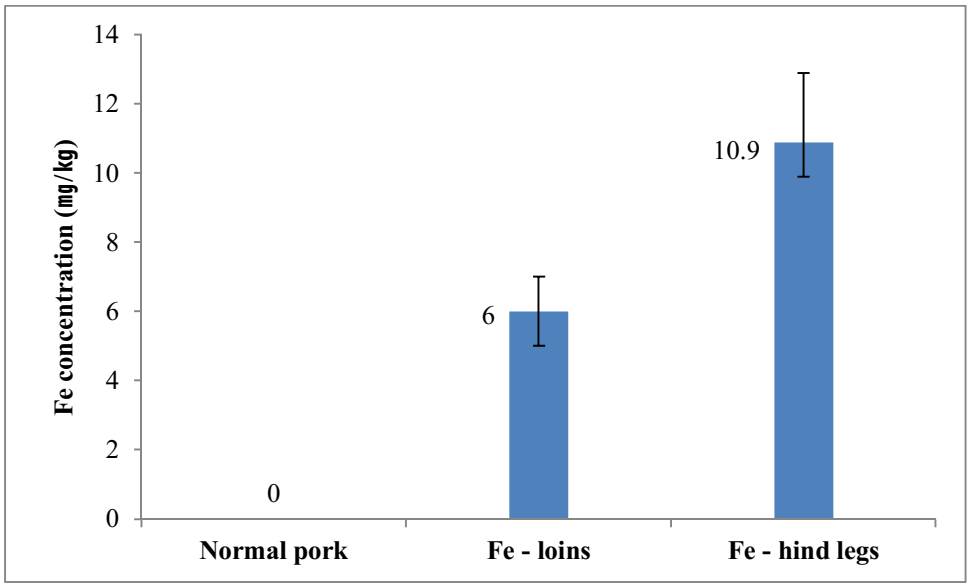


Figure 12. The concentration of iron in the loins and hind legs of a pig

Fe – pork meat was dried using vacuum dryer after quick freezing, so the concentration of iron in a same pork meat before drying was lower than these values.

The moisture content of pork meat was 66%.

Vertical bars represent the standard deviation of each data point(n = 3)

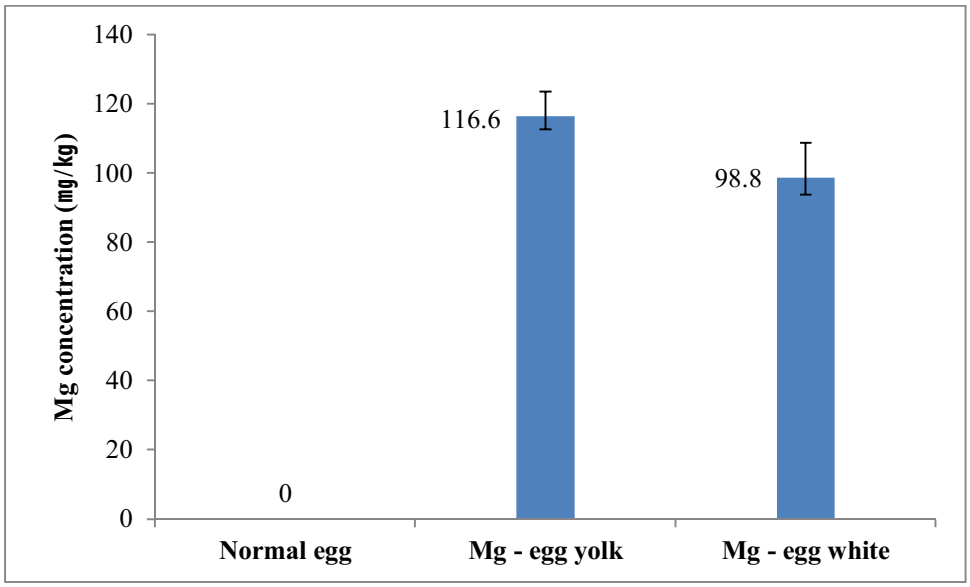


Figure 13. The concentration of magnesium in the egg yolk and egg white from the same egg

Mg - eggs were dried using vacuum dryer after quick freezing, so the concentration of magnesium in a same egg before drying was lower than these values.

The moisture content of egg was 75%.

Vertical bars represent the standard deviation of each data point(n = 3)

Ⅳ. Discussion

The normal procedure for culturing mineral-enriched yeast is as follows.

First, the fermenter and the medium are sterilized and inoculated with activated yeast seed. When the yeast growth reaches the log phase, sterilized additional nutrients and minerals are supplied in the fermenter. The yeast that is being cultivated actively transports the minerals together with the nutrients into the self [51]. When the fermentation is complete, the yeast cells are harvested by centrifugation and the unabsorbed residual minerals attached to the yeast cell's outer wall are washed with clean water. The culture filtrate and washing water are specially treated to prevent environmental pollution (Figure 14).

The typical operation of the fermenter requires a skillful technique to maintain optimum culture conditions for maximum yield. In addition, the volume of the fermenter is very large because it requires twice the volume of the operation. For example, to obtain a dry weight of 1,000 kg, if the fermentation yield is less than 10%, the volume of the operation must be 10,000 kg. Therefore, the volume of the fermenter should be over 20,000 kg. The fermenter is made of expensive parts because it has to be precise and a building is needed in which it can be installed. The cost of sterilizing the medium is also high. Thus, this procedure costs a lot of money.

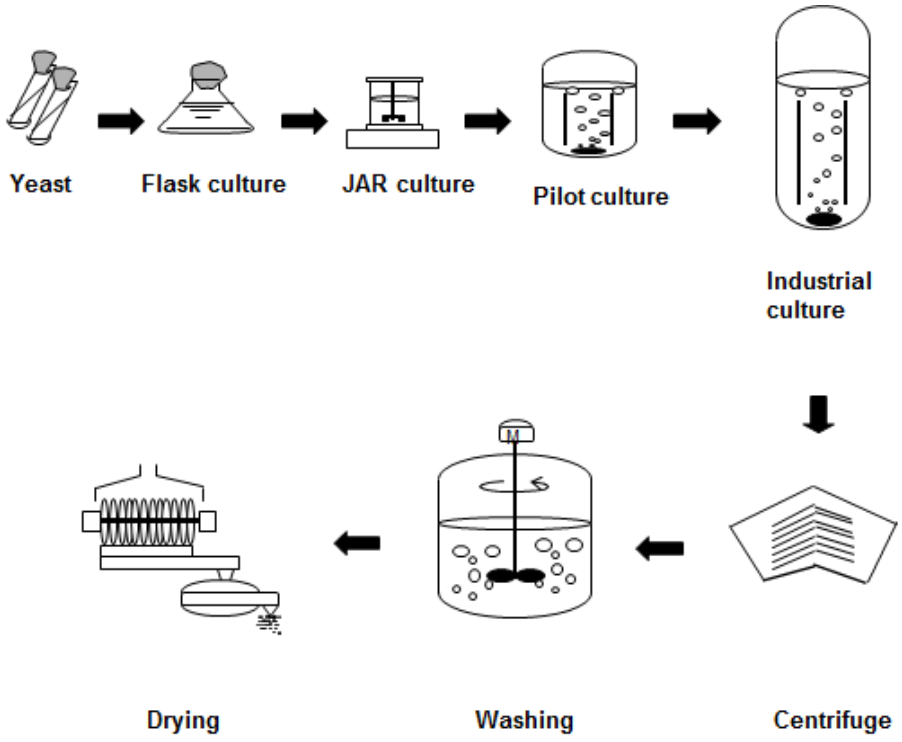


Figure 14. Schematic diagram of the traditional process of mineral-enriched yeast production

But, in producing mineral-enriched yeast, freeze-dried yeast can replace the yeast culture process. Therefore, an expensive fermenter is no longer needed. All that is needed is a mixer tank.

In addition, when producing mineral-enriched yeast, the use of freeze-dried yeast does not generate wastewater. By reducing the use of water and culture filtrate, centrifugation for cell harvesting is not required. It is expensive to dispose of wastewater because it is highly polluted. Therefore, the costs of wastewater treatment are not incurred and production costs are reduced by this new technique.

The washing process of mineral-enriched yeast generates wastewater, which reduces the effect of the production cost savings that were obtained by not creating culture filtrate. So the washing process also had to be eliminated.

In the process of producing chromium-enriched yeast, when the freeze-dried yeast met water, its life activity began, and the viscosity of the mixture was very high early on. But after 12 hours, the mixture's viscosity decreased because of the production of carbon dioxide and a small amount of ethanol by the yeast's metabolic activity. At this time, an additional 30% of the freeze-dried yeast weight was added into the mixture to absorb residual inorganic chromium. The lowered viscosity allowed for the mixing of the newly added yeast and the yeast was activated to absorb all of the residual inorganic chromium in the mixture tank.

After 24 hours, the yeast mixture was dried and the chromium content was measured as 700 ppm and no wastewater occurred in the production process. A repeat experiment that doubled the amount of chromium concentration obtained the same results. The concentration of chromium was 1,500 ppm (㎎/㎏). Other mineral-enriched yeast was produced using the same process. In conclusion, unless producing a very high content of mineral-enriched yeast, wastewater cannot be generated.

Mineral-enriched yeast could be used to develop agricultural products with higher mineral content.

Generally, both photosynthetic bacteria cell and cultured broths are used for crops, because these broths contain many useful metabolites. Organic selenium is absorbable through the pores of plant leaves, so it can be used by adding it into photosynthetic bacterial culture broth, which is highly effective for accumulating selenium. It should be noted that inorganic selenium has a low absorption rate through the pores of leaves, and high selenium concentrations cause leaf burning side effects.

Selenium-enriched yeast was used as the source of selenium, not NaHSe, Na_2Se_2, Na_2Se, $C_6H_5CH_2SeH$, or $(C_6H_5CH_2Se)_2$. To increase the efficiency of organic selenium, the cell walls of selenium-enriched yeast need to be degraded. But yeast cell walls are very rigid for enzymatic lysis and cell wall lysing

enzymes are expensive. So 5N hydrochloric acid was used for cell wall lysis and 5N sodium hydroxide to adjust to pH 7.0. This is a very effective method for obtaining a 100% organic selenium source. Additionally, yeast cell debris can be used as a medium for photosynthetic bacteria.

Spraying the culture of photosynthetic bacteria on crops is a more economical method than mixing it into the soil, and it is an environmentally friendly method that does not cause pollution from residual selenium in the soil [52].

New feed additives were developed with the mineral-enriched yeast produced by using freeze-dried yeast. Feeding it to livestock produced functional livestock products with increased mineral content.

Because more mineral-enriched yeast culture was added compared to the normal process, the new feed additives have many of yeast's useful components and the ripening progressed and the flavor improved until the drying process was finished.

Feed additives made from selenium-enriched yeast were fed to chickens to produce eggs and chicken with high selenium content [53].

The concentration of selenium in the egg yolk was higher than in the egg white. It is presumed that egg yolk exists in the body of a chicken longer than egg white.

Selenium content in eggs can be controlled by regulating the content of selenium-enriched yeast in feed additives. Selenium content is not usually measured in normal eggs.

Selenium content was measured evenly in the legs, wings, breasts, and skin of the chicken. It was not concentrated in a specific part.

The concentration of iron in pork produced with feed additives made from iron-enriched yeast was measured higher than in normal pork.

Eggs containing more than normal magnesium were produced by using the same method and the egg yolk had higher magnesium content than the egg white, as in the eggs containing selenium.

Functional farm products and livestock with increased mineral content by using mineral-enriched yeast is easy to eat by itself and is expected to be developed as functional processed food for children, pregnant women, and older adults.

In this study, mineral-enriched yeast was produced by a new method using freeze-dried yeast. It can replace the yeast culture step in the normal fermentation process. Because the process of the new method is simple, it is possible to reduce the costs of culture maintenance and the of fermentation apparatus and eliminate the occurrence of wastewater. The study's experiments confirmed that the theory of the new method is the same as that of normal cultures. Photosynthetic bacteria

cultures and feed additives were made from mineral-enriched yeast and functional foods with high mineral content were produced using the newly developed additives.

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ABSTRACT (Korean)

미네랄 함유 효모의 고효율 배양기술 기반 기능성 식품 개발

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문 기 혁

미네랄은 생체성분으로서의 무기질로도 정의되며, 무기영양소이다. 3 대 영양소인 당, 지방, 단백질을 보조해주는 단순한 구성성분일 뿐만이 아니라, 동물체내에는 존재하지 않으면 생명유지가 불가능한 필수원소들이다.

미네랄은 식품의 형태로 섭취하는 것이 가장 일반적인 공급의 방법이지만 토양 산성화로 인한 토양 내 미네랄 감소 등의 이유로 인간에게 충분한 공급이 되고 있지 않다. 그럼에도 불구하고 Fe, Zn, Cu, Mn, Co, I, Se, Mo, Cr, Mg 등의 11 종의 필수 다량원소와 15 종의 필수 미량원소는 반드시 생체에 공급되어야 하므로, 이들의 부족한 공급을 위한 인위적인 공급방식이 개발되어 왔다. 특별히 셀레늄, 철분, 아연, 크롬, 마그네슘과 코발트는 부족 시 결핍증상이 심각하다.

이들 미네랄은 특성상 체내에서의 흡수가 용이하지 않기 때문에 흡수가 잘되는 공급 원료와 공급 형태를 지속적으로 개발해 왔으며, 그 중 한 방법으로 효모 내에 미네랄이 고농도로 농축되도록 하는 방법이 개발되었는데, 발효방법으로 생산하여 안전하고, 식품형태이므로 선호도가 높다. 하지만 높은 효율로 배양하는 조건이 매우 까다롭고 배양여액과 세척 후 발생하는 폐수의 처리 비용 때문에 생산비용이 많이 발생하여 비싸다는 단점이 있다.

그래서 셀레늄, 철, 아연, 마그네슘, 크롬, 코발트 등을 함유하는 효모의 생산을 동결건조효모를 사용하여 고효율, 폐수발생이 없는 친환경적인 대량생산 하는 방법과 미네랄 함유 효모를 이용하여 미네랄 함량이 높은 기능성 식품을 개발하고자 하였다.

그 결과, 동결건조 효모를 사용하여 효모의 배양단계를 대체할 수 있었으며, 기존의 발효방법과 같은 원리로 생산됨을 실험을 통하여 확인 하였다. 미리 함량을 결정하여 생산하는 조건과 폐수가 발생하지 않는 조건을 확립하였고, 이를 통하여 배양장치 설치비용의 절감과 폐수처리비용을 낮추어서 고효율 생산을 할 수 있었다.

새로운 방법으로 생산한 미네랄 함유 효모는 광합성세균 배양액과 사료첨가제에 사용하여 유기태 미네랄이 함유된 새로운 농축산용 첨가제로 개발할 수 있었으며, 개발된 첨가제들을 벼, 배, 감, 파, 배추 등의 농산물과 닭고기, 계란, 돼지고기 등의 축산물 생산에 사용하여 미네랄 함량이 일반적인 제품보다 높게 함유된 기능성 식품을 생산하였다.

핵심되는 말: 미네랄, 셀레늄, 철분, 아연, 크롬, 마그네슘, 코발트, 효모, 광합성세균, 기능성식품