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Preparation of selenium enriched smearcase cheese and whey cheese

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SUMMARY

Selenium is a trace element essential for the body. Since Hungary is considered to be deficient in selenium and the utilization of selenium in the body is low, it is absolutely necessary to supplement it. One of the easiest ways for consumers to do so is to ingest it with basic foods. In the course of our research it was investigated whether it is possible to produce high selenium content smearcase (lump) cheese and whey cheese through the feeding of cattle. During this, when supplementing the feed of cattle with 1-2 mg Se/day of yeast daily, the selenium content of milk increased from 18 μg/kg to 31 μg/kg as a result of a supplementation of 1 mg, and to 53 μg/kg as a result of supplementation of 2 mg. The selenium content of smearcase cheese produced from the latter was measured to be 138.1 (66.0) μg/kg, while that of whey cheese was 167.2 (80.8) μg/kg. Control values are shown in parentheses. It was found that the smearcase cheese cheese and whey cheese produced from the milk obtained as a result of a selenium supplementation of 2 mg nearly two and a half, three times more selenium than those produced from milk without selenium supplementation.

Introduction

Selenium is a mineral that is essential for our body to function and is present in almost all of our cells **[1]**. It is an antioxidant in its own right, but also plays a vital role in the action of the glutathione peroxidase enzyme, which acts as a protective agent against toxins entering the body. It provides protection against harmful free radicals, thus playing an important role in preventing certain types of cancer **[2]**. It also reduces the likelihood of developing cardiovascular disease, liver damage, and the risk of developing cataracts and, at appropriate concentrations, also has a beneficial effect on most elements of the immune system **[3]**.

According to literature data, the utilization of selenium in the body is low. The reasons for this are that its absorption from the digestive tract is limited, most of the selenium-containing compounds absorbed are excreted in the urine, and the amount remaining in the body is utilized to a limited extent **[4]**. Due to the above reasons and to Hungary's limited selenium supply, it is necessary to supplement this mineral. There are two options for selenium supplementation: on the one hand, dietary supplements, and on the other hand, the consumption of foods with increased selenium content. The topic of our paper can be significant in both animal and food science, as dairy products are part of our daily diet and therefore can serve as an essential source of selenium.

Thus, selenium is an essential trace element for the body, and the significance of its role has increased greatly due to research carried out in recent decades. In the 1930s, it was still believed to be a toxic heavy metal that, in higher doses, would cause the death of a living organism. In 1943, its carcinogenic property was already described **[5]**. A few years later, selenium supplementation was shown to reduce the incidence of cancer **[6]**, and the essential role of selenium was first published in 1957, when animal studies had shown that selenium added to food prevented liver necrosis **[7]**. Since 1966, we can also read about its anti-cancer effect **[8]**.

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In our environment (in soil, water, and in all living organisms), selenium occurs in oxidation numbers -2 (selenide), +4 (selenite) and +6 (selenate). Selenates and selenites are water-soluble compounds, and are therefore most commonly found in waters in these forms **[9]**. In addition to its inorganic compounds, its organically bound forms are also known, in which selenium is present as selenide. These are mostly seleno-amino acids and their derivatives. Foods of plant origin contain selenomethionine, while foods of animal origin contain selenomethionine, as well as selenocysteine. Selenomethionine is essential for both humans and livestock since it cannot be produced from inorganic sources, but selenomethionine can be converted into selenocysteine in the body **[10]**.

Selenium has been shown to play a significant role, directly or indirectly, in many physiological processes. Its absence can cause the development and worsening of many diseases, such as heart and brain catastrophes, cancers, thyroid dysfunction, spread of viruses (influenza, HIV, Ebola) and reproductive problems **[11]**.

The most important role of selenium is due to its antioxidant effect, which is expressed through its attachment to various enzymes. It plays a key role in the action of the glutathione peroxidase enzyme, which, by reaction with hydrogen peroxide and other harmful lipid and phospholipid hydroxides, prevents the formation of harmful free radicals, inhibits DNA damage and the formation of metabolically active carcinogens **[12]**. Selenium is incorporated into the enzyme as selenocysteine, occupying the place of sulfur. In the body's defense against oxidation, the biochemical property that ensures the importance of selenium is that it is easier to reduce than sulfur **[13]**.

Selenium is present in the human body in quantities of 10 to 15 mg. It is present in almost all of the cells of our body, but most of it accumulates in the kidneys, liver, spleen, pancreas and testes. According to the Hungarian Food Codex, the recommended daily intake (RDA) for adults is 55 µg/day **[14]**. According to a 1996 report by a panel of WHO, FAO and IAEA experts, 400 µg Se/day is the limit value above which the adverse effect of selenium are to be expected **[15]**.

The selenium content of foods varies widely, but our foods usually contain only small amounts. The selenium content of the Brazil nut (*Bertholletia excelsa*) of South America has the highest selenium content; it may contain more than 100 **µg** per piece **[16]**. The richest sources of selenium among our foods are animal internal organs and seafood, followed by animal meat. Since the selenium content of foods taken as part of the daily diet is insignificant, the selenium intake cannot be increased by increasing food consumption. Our selenium needs can be met either by dietary supplements, or by selenium-enriched foods. Dietary supplements have been used to supplement nutrients and physiologically active substances that are body needs since the 1980s. Today, these products are available in the form of highly utilizable capsules or pills **[17]**, containing mainly selenite, selenate, selenomethionine or selenium-enriched yeast.

In functional foods enriched with selenium, selenium is present in its natural or near-natural form. In the production of such foods, manufactured using sophisticated technologies, selenium is given to the plant or animal as a dietary supplement, which, when absorbed, reaches its natural state through a series of biochemical reactions. During the transformations, the oxidation state of the selenium may change, therefore, it is important to monitor the form in which the nutrient of plant or animal origin contains it **[18]**.

Despite the decline in milk consumption, milk as a staple food is still considered to be the source of selenium for humans. The selenium content of milk is 25 µg/l on average, so milk and dairy products constitute 6 to 10% of the daily intake of selenium **[19]**. Selenium added to cattle feed offers a possibility to increase the selenium content of milk. Selenium supplementation can be accomplished by adding either inorganic sodium selenite or organic selenomethionine, selenocysteine or selenium-enriched yeast. However, when selecting the additive, it should be kept in mind that selenite may be reduced to insoluble selenide or elemental selenium in the rumen of ruminant animals. It is advisable to prefer the organic from, because its absorption is more efficient and it is less toxic than the inorganic form **[20]**.

Material and methods

Experimental parameters, sampling

In the experiment, three Simmental type cattle were included, which is raised for both milk and meat and is the ancestor of the Hungarian spotted cattle. Each of the three experimental animals were approximately 5 months pregnant, in the same phase of lactation, with an average milk production of 4,000-5,000 liters during the milking period, fed on hay and corn silage. During the experiment, the selenium content of the basic feed was measured and selenium supplementation was performed using selenium-enriched yeast. The daily feed contained 0.42 mg selenium. Selenium-enriched yeast was added to the feed for two weeks at a rate of 1 mg/cow/day, then for two more weeks at a rate of 2 mg/cow/day (***Table 1***).

Cattle milk was sampled once every week of the experiment. During the first two weeks, when no selenium supplementation was performed, control samples were collected. For the measurements, samples from the cows were taken from the total amount of milk obtained, and they were stored frozen at -18 °C until further processing. The element content investigated by us was not affected by freezing.

Dairy product preparation

Smearcase cheese and whey cheese were prepared from the control and from the milk obtained from cattle that received 2 mg of selenium supplementation at the dairy plant of the Food Industry Innovation Center of the Institute of Food Technology of the University of Debrecen.

Preparation of smearcase cheese

For the preparation of smearcase cheese, 10 liters of raw cow's milk was used per sample. Control and selenium-enriched milk were heat treated at 65 °C for 30 minutes before use, then they were cooled back to 36-38 °C. The proper amount of Presure Simple Brun rennet (5 grams/liter**)** was added to the cheese milk, it was mixed thoroughly, and then it was kept at the same temperature for 40 minutes without stirring. The end of the coagulation process was marked by the fact the separation of milk from the wall of the coagulation vessel, and by breaking like china. At this point, the curd was cut with a cheese harp to approximately bean-sized pieces and it was agitated to remove some of the excess whey from the lumps. Following this, the cheese crumbs were collected into a plastic cheese mold, it was dried for 24 hours, rotating it every half hour, then matured for a further week in a refrigerator at high humidity. Cheese lumps and fresh cheese wheels are shown in ***Figures 1*** and ***2***.

Preparation of whey cheese

For the preparation of whey cheese, the whey remaining in the cheese-making process was collected, it was poured into a suitable container and was brought to a boil. When the temperature of the whey reached 94-95 **°C**, a tablespoon of citric acid was added and the fluffy precipitate, whey protein was filtered through a cheesecloth. The product was stored in a refrigerator at 4 **°C** until the measurement.

Determination of selenium content

Samples were prepared for selenium analysis by a two-stage digestion. 2.00±0.01 grams of sample were weighed into each digestion tube. For pre-digestion, 10 cm^3 of concentrated HNO_3 was added to the samples, which were then allowed to stand overnight and then heated in a block digester at 60 °C for 30 minutes. For the main digestion, 3 cm^3 of 30% H_2O_2 was added, the samples were once again placed in the block digester and kept at 120 °C for 90 minutes. Following this, the samples were cooled to room temperature, their volume was adjusted to 50.0 cm^3 with deionized water, and they were filtered through 'Filtrak 388' filter paper.

An inductively coupled plasma mass spectrometer (ICP-MS, *Thermo Scientific X-1 Series 2*) was used for the measurement of selenium content. For the determination, calibration solutions were prepared with deionized water for metal analytical measurements. The calibration curve ranged from a concentration of 0.0 to 100 µg/kg. The linearity of the 11-point calibration curve was r^2=0.9999. The limit of detection was 0.06 µg/l. For the calibration, a 100 µg/l working solution was prepared from a 1 g/l selenium reference stock solution (Scharlau Cat. No. SE00110500), and it was stored in a refrigerator at 4 °C. On the days of the measurements, calibration solutions with concentrations of 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 50 and 100 µg/L were prepared from the working solution. Measurements of the digested samples were performed in triplicate. Plasma temperature was 6,000 °C.

Statistical analysis

Statistical analysis of the measurement data obtained from the three parallel analyses was performed using the SPSS 20.0 (IBM SPSS Statistics, 20.0) program. The significance level was set to 5% in all cases. Significant differences between the groups were assessed by the least significance difference (LSD) test.

Results and evaluation

The selenium content of milk samples was significantly increased by the selenium supplementation of the feed. The mean values of our test results obtained from three replicate analyses are shown in ***Table 2*** and ***Figure 3***.

The statistical analysis revealed that there was a significant difference between treated and control, i.e., untreated cow's milk; compared to the control group, the amount of selenium in milk increased as a result of selenium administration. Compared to the control milk, the amount of selenium increased by 73% by week 3-4 of the experiment, and by a further 71% by week 5-6, so with a supplementation of 2 mg Se/cow/day, the milk contained almost three times more selenium than the milk of cows consuming only basic feed.

After heat treatment, fresh cheese was prepared from the milk taken during the control period and after the administration of the highest dose of selenium, and whey cheese was prepared from the whey remaining after cheese production. Dairy products were prepared at the practice dairy plant of the university, and the element content was also determined on campus.

Analytical results of the products manufactured by us are illustrated in ***Figure 1***. Results of the control group are shown in green, while results of the products made from the milk of cows receiving the selenium treatment are shown in yellow. The horizontal axis (x) shows the name of the dairy products, while the vertical axis (y) shows the selenium content. It can be seen that selenium is enriched in both smearcase cheese and whey cheese as compared to whole milk. There is a significant difference between the results of products made from the control and treated milk. This proved that high selenium content dairy products can be prepared from selenium-enriched milk, some of the selenium added to the feed appears in the milk and in dairy products.

In the case of whole milk, the selenium content increased from 18 μg/kg to 53 μg/kg as a result of a selenium supplementation of 2 mg. This milk served as the basis for our dairy products. The smearcase cheese prepared from the milk of treated cows contained 200.0 μg/kg selenium, while the control contained only 88.6 μg/kg. The trend was similar in the case of whey cheese; the selenium content of the product made from selenium-enriched milk was 167.2 μg/kg, while the whey cheese prepared from the milk of cows with no selenium supplementation contained only 80.8 μg/kg.

Conclusions

Dairy products are part of our daily diet, and selenium is an essential trace element for our body, the absence of which may lead to the development of various diseases. Hungary is one of the areas that is deficient in selenium, so it would be advisable to improve the selenium status of the population. In our research, we worked on the development of a product or product group through the consumption of which the health protection effect of selenium can manifest itself. Increasing the selenium content of milk and dairy products seemed to be an obvious solution for us, since this product group is consumed by a large part of the population, so this way they may have access to this important essential trace element.

In our studies, cattle were fed with feed containing selenium levels elevated by 1-2 mg, and the development of the selenium level of milk was observed. A dose of 2 milligrams of selenium per day was found to be safe for the animals, so milk obtained while administering this dose was chosen for the production of food intended for human consumption. In the course of our experiments, the selenium content of milk increased from 18 *μg/kg* to 31 μg/kg as a result of a selenium supplementation of 1 mg, while it increased to 53 μg/kg as a result of a supplementation of 2 mg. From the milk smearcase cheese and whey cheese were prepared. In the case of smearcase cheese, a selenium content of 88.6 μg/kg was measured in the cheese prepared from the milk of untreated cows, while the value was more than twice as high, 200.0 μg/kg in the case of the selenium-enriched feed. Similarly, in the case of whey cheese, the selenium content increased from 80.8 μg/kg to 167.2 μg/kg. It was found that the smearcase cheese and whey cheese prepared from milk obtained after a selenium supplementation of 2 mg contained two and a half, three times more selenium than the products prepared from milk without selenium supplementation.

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