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SELENIUM EFFECT ON RYE MALT QUALITY

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*Rye (*Secale cereale *L.) is the most important cereal crop after wheat, rice and maize. A substantial part of the rye yield is used for bread making, especially in European countries. There have been numerous studies on grain enrichment with selenium (Se), as it is known that selenium is a fundamental trace element essential for human health and in the form of selenoproteins plays key structural and enzymic roles. The main aim of this study was to investigate the effect of different selenium concentrations on quality indices of rye malt — the content of malt extract, diastase activity, selenium and total phenol content in malt. Rye grain of 95% viability was soaked and germinated at temperature +6 ± 2 °C for three days +18 ± 2 °C, using sodium selenate Na_2SeO_4 solutions (Se concentration 3 mg l^{-1}, 5 mg l^{-1}, 10 mg l^{-1}), and dried in an oven for 24 hours at temperature +70 – 112 °C. Germination of grain with deionised water served as a control. The obtained results showed that an increase of selenium concentration caused increase of malt extract concentration (from 74 to 80%), selenium concentration (from 0.0139 to 0.3251 mg kg^{-1}) and total phenol concentration (from 3.13 to 3.63 mg GAE g^{-1} DW) in rye malt, while diastase activity decreased from 330 to 216.*

Key words: *selenium, rye, malt extract, diastase activity, total phenols.*

INTRODUCTION

Rye (*Secale cereale* L.) is the most important cereal crop after wheat, rice and maize. A substantial part of the rye yield is used for bread making, especially in European countries (Michalska *et al*., 2008). Rye malt has amazing healing properties. It was well known to our ancestors. Porridge, bread drink and soup made from rye malt were actively used for human consumption as an effective means of winter-spring beriberi; treatment of exhausted long serious illness; and during farming work, raised strength and endurance (Смирнова и др, 1989). Rye malt is widely used in bread production today. Rye malt contains a much active enzyme (diastase) and is used for promotion of rye bread scald candying (Aly *et al*., 2004). Rye malt is a natural food product that is made from the best varieties of rye with a coefficient of germination not lower than 96%. Its nutritional value lies on high content of protein, oligopeptides; digestible polysaccharides (glucose, maltose, dextrins), polyunsaturated fatty acids, minerals, vitamins (Michalska and Zieliński, 2006; Donkor *et al*., 2012).

The microelement selenium is needed for normal function of the human body, as it is a constituent of some enzymes and hormones, interacts with vitamins, participates in oxidising processes, and metabolism of proteins, carbohydrates, and fats (Duma *et al*., 2011). Selenium occurs in enzyme glutathione peroxidase, the main part of the antioxidative defence system in living cells (Lyons *et al*., 2005). Therefore, selenium and its compounds have notable antioxidative properties (Surai, 1999). The selenium content in foodstuffs depends mainly on its content in plant and animal raw materials, and this is affected by the content of selenium in the soil.

It has been reported that cereals and cereal products have a wide range of selenium concentration, between 0.0010 and 0.550 mg kg^{-1} (Anonymous, 2001).

Germination of grains improves the nutritional value of grain — the content of protein, amino acids, and vitamins as well as activity of enzymes are changed. It is known that germination may influence the level and bioavailability of the bioactive compounds (Lintschinger *et al*., 1997; 2000; Katina *et al*., 2007; Tian *et al*., 2010).

There have been numerous studies that showed the effects of selenium (Se) on wheat, barley and oats sprouting activity and on raising concentration of biologically active substances and vitamins in germinated grain (Dūma, 2010).

The aim of this study was to investigate the effect of different selenium concentrations on quality indices of rye malt — malt extract, total phenols and selenium concentration, and diastase activity, in rye malt.

MATERIALS AND METHODS

Plant material. The research object was rye grain (variety 'Kaupo') from Ltd. "Naukšēni", harvested in 2011. Rye grain were soaked and germinated at temperature +6 ± 2 °C for three days, using sodium selenate Na_2SeO_4 solutions. The concentrations of selenium used were was 3, 5, and 10 mg l^{-1}. Germination of grain with deionised water served as a control. After germination, all sprouts were dried for 24 h at a temperature of +70 – 112 °C; then they were ground. Moisture of malt samples ranged from 7.10% till 8.97%.

Determination of Se in germinated rye grains. The selenium concentration was determined by standard method AOAC 996.16, based on wet digestion with nitric and perchloric acids, reaction with 2.3-diaminonaphtalene (DAN) reagent and fluorimetrical determination at excitation wavelength of fluorimeter at 375 nm and emission at 525 nm.

Determination of malt extract concentration. Concentration of unfermented malt extract was determined by hot extraction method.

200 ml of 47 °C warm distilled water was added to 50 g ground malt, maintained for 30 minutes at 45 °C in a water bath regularly stirring, then heated to 70 °C, and 100 ml distilled water at 70 °C was added. Stirring constantly for 1 hour, distilled water was added till 450 ml, mixed, and followed by filtering. The density of filtrate was determined and the mass of extract e, % was found.

Concentration of malt extract was calculated on the the basis of air-dried substance E_1 %, as follows:

$E_1 = \frac{e(W+800)}{100-e}$, where

e – extract mass of the filtrate, %;

W – malt moisture, %

Determination of diastase activity with Phadebas. The unit of diastase activity, the Gothe unit, is defined as that amount of enzyme that will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 °C under the conditions of test. Results are expressed in Gothe units per gram of malt (Sak-Bosnar and Sakac, 2012).

The diastase activity of samples was measured by the Phadebas method (Sak-Bosnar and Sakac, 2012). A tablet of an insoluble blue-dyed, cross-linked starch was used as the substrate for the degradation reaction. After dissolving 1.00 g of malt in acetate buffer in a volumetric flask, 5.0 ml of malt solution was transferred to the test tube and incubated in a water bath at 40 °C for a few minutes. A blank was prepared by adding 5.0 ml of acetate buffer solution and then treated in the same manner as sample solutions. After placing Phadebas tablets into both test tubes, a timer was started. The tubes were quickly removed from the water bath, stirred and then returned to the water bath. After 30 min, the reaction was terminated by adding 1.0 ml 1 M sodium hydroxide solution. The mixture was stirred again and filtered. The absorbance of the sample was measured at 620 nm with deionised water as a reference. The absorbance of the blank was subtracted from that of the sample solution ($_{DA620}$). The diastase activity, expressed as DN or diastase number, was calculated from the absorbance measurements, respectively:

$DN=28.2\times\Delta A_{620}-2.64$

Diastase activity was referred to as DN in the Schade scale, which corresponds to the Gothe scale number, or g, of starch hydrolysed per hour at 40 °C per 100 g of malt.

Determination of total phenol concentration (TPC). Total phenol determination started with preparation of extracts from rye malt. Rye malt was finely ground in a laboratory mill CIATRONIC KSW 2669. Four grams of ground samples were extracted 10 min in an ultrasound bath (ULTRASONS, SELECTA P) with 40 ml of solvent (7/7/6 ethanol/acetone/water (v/v/v) mixture). After centrifugation at 3000 min^{-1} for 10 min using a centrifuge MEDITRONIC BL-C, the supernatant was removed and the extraction was repeated once more. The supernatant was collected in a 50 ml volumetric flask and refilled with solvent to previous volume. The TPC of the malt extract was determined according to the Folin-Ciocalteu spectrophotometric method with some modifications (Singleton *et al*., 1999). First, 0.25 ml of sample was transferred to a 25.0-mL volumetric flask containing 6 mL H_2O, to which 1.25 mL of undiluted Folin-Ciocalteu reagent were subsequently added. After 1 min, 3.75 ml of 20% aqueous Na_2CO_3 was added, and the volume was made up to 25.0 ml with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h incubation at 25 °C, absorbance was measured at 760 nm using a spectrophotometer JENWAY 6300 (Dabiņa-Bicka, 2011). Total phenol concentration was expressed as gallic acid equivalents (GAE).

Statistical analysis. The statistical analyses of data were carried out using Microsoft Excel for Windows 7.0 (Microsoft Corporation, Redmond, WA). Mean value, standard deviations and significant values were calculated. *P*-values < 0.05 were regarded as significant.

RESULTS

The selenium concentration in rye malt differed significantly ($P < 0.05$) between germination solutions with different selenium concentrations (Fig. 1). The concentration of selenium in rye malt using solution where selenium concentration was 3 mg l^{-1} was 5.4 times higher than in the control. Accordingly, when the concentration of selenium in solution was 5 mg l^{-1} the increase in grain was by 11.7 times. At a selenium concentration of 10 mg l^{1}, selenium uptake increased by 22.4 times.

The influence of different selenium concentrations on the content of rye malt extract is shown in Figure 2. All Se concentrations used promoted increase of rye malt extract concentration, and the highest value was observed when the selenium concentration was 5 mg l^{-1}. In this case, the

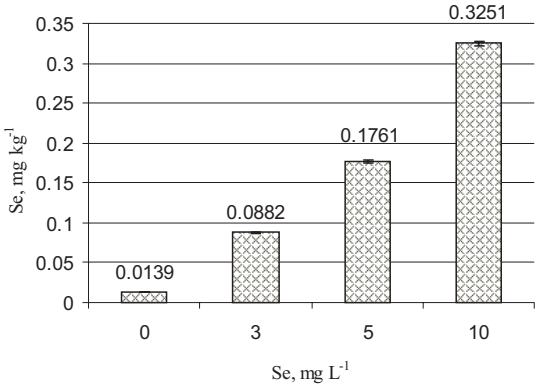

Fig. 1. Se concentration in malt, depending on the concentration of Se in solution.

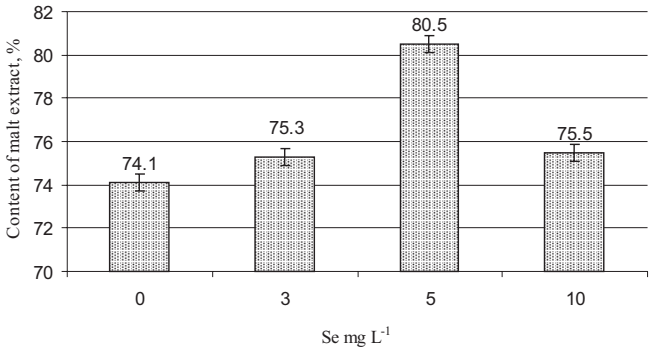

Fig. 2. Content of rye malt extract depending on Se concentration.

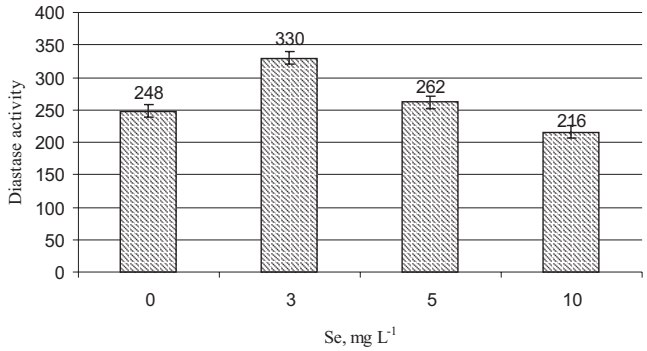

Fig. 3. Diastase activity (Gothe units) in rye malt depending on Se concentration in solution.

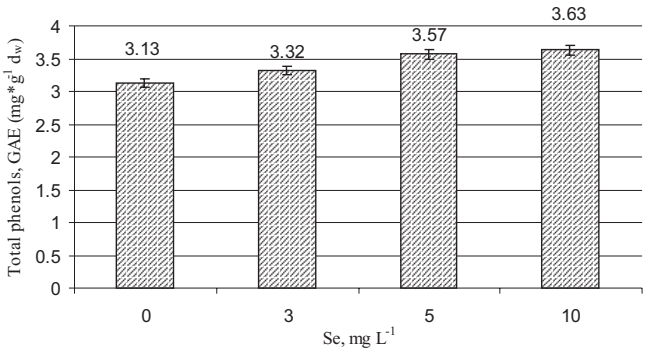

Fig. 4. Total phenol content in rye malt depending on Se concentration.

concentration of malt extract increased by 6.4%, compared with the control sample. There is an insufficient amount of information in the literature about the effect of selenium on concentration of rye malt extract.

Change of diastase activity in rye malt in relation to Se concentration in solution are shown in Figure 3. Rye malt diastase activity depended on selenium concentration in solution — when selenium concentration was 3 mg l^{-1} and 5 mg l^{-1}, diastase activity increased by 33% and 5.6%, respectively. At a selenium concentration of 10 mg l^{-1} rye malt diastase activity decreased by 12.9%, compared with the control sample.

The use of selenium treatment also significantly increased ($P < 0.05$) the total phenol concentration (Fig. 4). When concentration of selenium was 3 mg l^{-1}, the content of total phenols increased by 6.1%, at concentration 5 mg l^{-1} the increase was by 14%, and when 10 mg l^{-1} the concentration of total phenol increased by 16%, compared with the control sample.

DISCUSSION

It is known that cereals belong to Se non-accumulating plants due to a limited ability of selenium uptake from the soil and incorporation in compounds (Terry *et al*., 2000). Selenium concentration in wheat, hull-less barley, hull-less oat and rye grain harvested in Latvia is within the range from 0.01533 mg kg^{-1} to 0.03533 mg kg^{-1}, in addition, the variety of cereal has a significant effect on the content of selenium ($P < 0.05$). The concentration of selenium is lower in hull-less grain (barley and oats) (Dūma, 2010). In comparison, the concentration of selenium in cereals cultivated in Finland is five times, and in Germany 25 times higher than in Latvia (Eurola, 1990; Combs, 2001). The obtained results showed that all analysed Se (VI) concentrations promoted uptake of selenium in rye malt. With increasing Se concentration in solution, the concentration of selenium in malt also significantly ($P < 0.05$). increased. This allows to conclude that selenium accumulation in malt is proportional to its concentration in solution and it is practically linear. These results are similar to those found previously (Lintschinger *et al*., 1997)

The most important parameter in the evaluation of the malting usability of rye grain is concentration of malt extract, estimated as the proporion of extractive substances obtained from malt produced from a given grain under optimal technological conditions. Concentration of malt extract varies in wide range, primarily depending on grain quality (Błażewicz *et al*., 2007). Concentration of malt extract in unfermented malt can be up to 80% (Смирнова и др., 1989). Variability in concentration of malt extract may be due to diversified water utilisation, affecting the yield and protein content of grain as well as efficacy of agricultural practices (Błażewicz *et al*., 2007).

Malting includes germination and drying of cereal seeds and its prime aim is to promote the development of hydrolytic enzymes that are not active in raw seeds. The main enzymes produced during germination are starch hydrolases (Yamasaki, 2003; Traoré *et al*., 2004). Diastases are a group of starch-digesting enzymes including α- and β-amylase (Sak-Bosnar and Sakac, 2012). The amount of enzyme depends on various factors, such as type of cereal, environment, duration of germination and temperature. For example, the diastase activity of barley malt has been estimated to be about 190 CU/g. (Helland *et al*., 2002; Mark *et al*., 2008; Kádár *et al*., 2011).

Plant phenolics, including, flavonoids and phenolic acid and tocopherols, are known to protect plants against tissue injuries, high levels of oxygen, free radicals and reactive oxygen species formed by the by-products of photosynthesis. These molecules also play an important role in the protection of food against lipid oxidation and in human health, by counteracting the risk of cardiovascular diseases, cancer and cataract, among other degenerative diseases of aging (Bondia-Pons *et al*., 2009). The concentration of total phenol in barley malt varieties has been reported to range from 2.51 to 3.45 mg GAE g^{-1} DW (Dabiņa-Bicka, 2011).

In conclusion, the concentration of selenium in rye malt increased with increasing concentration of selenium in water solution by 5.4 times (3 mg L^{-1}) to 22.4 times (10 mg L^{-1}). All analysed selenium concentrations increased the concentration of rye malt extract and the highest results were obtained using selenium concentration 5 mg L^{-1}. The activity of rye malt diastase is in inverse ratio to the concentration of selenium in solution. The concentration of total phenol increased with increasing concentration of selenium.

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SELĒNA IETEKME UZ RUDZU IESALA KVALITĀTI

Rudzi (*Secale cereale* L.) ir viena no svarīgākajām labības kultūrām pēc kviešiem, rīsiem un kukurūzas. Eiropas valstīs rudzus plaši izmanto maizes ražošanā. Pasaulē ir veikti vairāki pētījumi par graudu bagātināšanu ar selēnu (Se), bet ir maz pētījumu par selēna ietekmi uz rudzu iesala kvalitāti. Ir zināms, ka selēns ir mikroelements, kuram ir būtiska loma cilvēka veselībā, pie tam selēns ir daudzu selēnproteīnu vai selēnenzīmu svarīga sastāvdaļa. Pētījuma galvenais mērķis bija izpētīt selēna ietekmi uz ekstraktvielu daudzumu, diastāzes aktivitāti, selēna un fenolu saturu rudzu iesalā. Rudzu graudi ar 95% dzīvotspēju tika diedzēti trīs dienas +6 ± 2 °C temperatūrā dažādu koncentrāciju Se šķīdumos (3 mg L^{-1}, 5 mg L^{-1}, 10 mg L^{-1}), un kaltēti 24 stundas +70 – 112 °C temperatūrā. Kontrole — graudi tika mērcēti dejonizētā ūdenī. Iegūtie rezultāti parādīja, ka, palielinoties selēna koncentrācijai šķīdumā, palielinās iesala ekstrakta saturs (no 74 līdz 80%), selēna (no 0.0139 līdz 0.3251 mg kg^{-1}) un fenolu (no 3.13 līdz 3.63 mg GAE g^{-1} DW) daudzums rudzu iesalā, bet diastāzes aktivitāte samazinās no 330 līdz 216 vienībām.