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The influence of different copper concentrations on barley grain sprouting and the content of total phenols

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Barley is a major world crop ranked as the fourth most important cereal in terms of plant area. Barley is consumed around the world, mostly in the malted form in brewing industry. Recently, many investigations in malt production intensification with biologically active compounds or ferment preparations have been accomplished. However, comprehensive studies on the effect of copper additives on barley grain sprouting are still required. The aim of this study was to explore the influence of different copper concentrations on barley grain sprouting and on the content of total phenols. Barley grains of 92 % viability were soaked in different solutions (Cu^{2+} concentration 10–500 mg/l) for 12 hours, left for sprouting at a temperature of 18 ± 2 °C for 5 days, and then dried in the oven for 48 hours at 50 °C. The control sample of grain was soaked in deionized water. The sprouting activity of the grain was determined after sprouting, whereas the content of total phenols was determined after the grain had dried out. The obtained results show that at the copper concentration of 10–50 mg/l, barley grain sprouting activity increases essentially. At the copper concentration of 100 mg/l, barley grain sprouting decreases sharply; moreover, at the copper concentration of 500 mg/l no barley grain sprouting activity was detected. The highest content of total phenols was observed when copper concentration in the solution was 50 mg/l.

Keywords: copper, barley, sprouting activity, total phenols

Introduction

Barley is a major world crop ranked as the fourth most important cereal in terms of plant area. Barley is consumed around the world, mostly in the malted form in brewing industry [1]. Barley malt is the dried product of barley germinated in controlled conditions. It is widely used in beverages and food industry [2]. The types of beer determine such indices as the taste, aroma and flavour of malt; therefore, malt production is very important in brewing industry. Malt acquires its properties exactly during the sprouting process; therefore, the quality of malt depends on the quality of grain.

About 80 % of phenolic compounds present in beer are derived from barley malt, and the rest comes from hop. They contribute to astringency and colour, serve as browning substrates, participate in chill haze formation and are responsible for the overall beer stability. Malt contains various compounds of barley (endogenous phenolic compounds) and from the malting process (the Maillard reaction products), which can play a significant role in malting and brewing through their antioxidant properties [7].

Recently, many investigations on malt production intensification with biologically active compounds or ferment preparations have been accomplished. However, comprehensive studies on the effect of microelement additives on barley grain sprouting are still required.

Micronutrients are involved in all metabolic and cellular functions [3]. Copper is an essential micronutrient involved in a variety of biological processes indispensable for sustaining life [4]. In physiological conditions, copper exists in two oxidation states – Cu^{1+} and Cu^{2+} – and can interchange these forms (monovalent copper is unstable). This allows copper to function as a reducing or oxidizing agent in biochemical reactions [3]. At the same time, it can be toxic when present in excess, the most noticeable chronic effect being liver damage [5].

Copper functions as a cofactor and is required for structural and catalytic properties of a variety of important enzymes, including cytochrome *c* oxidase, tyrosinase, *p*-hydroxyphenyl pyruvate hydrolase, dopamine beta hydroxylase, lysyl oxidase, and Cu-zinc superoxidase dismutase (Cu, Zn-SOD). These enzymes are involved in an array of biological processes required for growth, development, and maintenance [3, 5].

The aim of this research was to investigate the influence of different copper concentrations on barley grain sprouting and the content of total phenols.

Materials and methods

Plant material

The research object was barley grains (variety "Class") from "Tērvete" Ltd. (harvested in 2011). Barley grains were soaked and germinated at room temperature (18 ± 2 °C), in natural day/night conditions using copper sulphate solutions prepared from $CuSO_4 \cdot 5H_2O$. The period of germination was 120 h. The copper concentration was 10, 50, 100 and 500 mg/l. The grain (100 pieces) was soaked for 12 h in the above-mentioned solutions (500 ml) and let to sprout. During germination, the sprouts were rinsed four times every day with 100 ml of corresponding solutions. Grain germination with deionized water served as the control. After germination, all sprouts were dried for 48 h at 50 °C (till moisture 7 ± 1 %) and then ground. The experiments were

perfomed in ten replications. Barley grain viability was determined before grain germination. The sprouting activity of the grain was determined after sprouting, whereas the amount of total phenols was determined after grain had been dried out.

Grain viability

To assess grain viability, the tetrazolium test was used [8]. It relies on the reduction of a tetrazolium salt (which in its oxidized state is colourless) to an insoluble red compound (formazan), in the presence of dehydrogenase activity (indicative of cell viability), thus staining the respiring tissue; 100 grains were cut along their main axis and placed in Petri dishes with the cut surface submerged in a 1 % tetrazolium salt solution (2,3,5-tripheniltetrazolium chloride) and kept at 25 °C for 4 h. The embryos in which at least 30 % of their surface was stained red were assumed to be viable. The experiment was conducted in four replications.

Grain sprouting activity

Standard sprouting activity tests were conducted on all samples according to the between-paper method of the International Seed Testing Association (ISTA) [6]. The percentage of sprouted grain was determined after five days. Grains were visually assessed according to the ISTA rules. The experiment was conducted in ten replications.

Total phenol content (TPC)

Total phenol determination starts with the preparation of extracts from barley malt. Barley malt was finely ground in a laboratory mill (CIATRONIC KSW 2669). Four grams of ground samples were extracted after 10 minutes in an ultrasound bath (ULTRASONS, SELECTA P) with 40 ml of a solvent. To reach a compromise between alcoholic and acetone extractions, a 7/7/6 ethanol/acetone/water (v/v/v) mixture was tested. 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 the solvent to the mark. The TPC of the barley and malt extract was determined according to the Folin–Ciocalteu spectrophotometric method with some modifications [6]. First, 0.25 ml of a sample was transferred to a 25.0 ml volumetric flask containing 6 ml of H_2O, to which 1.25 ml of the undiluted Folin–Ciocalteu reagent was subsequently added. After 1 min, 3.75 ml of 20 % aqueous Na_2CO_3 was added, and the volume was supplemented to 25.0 ml with H_2O. The control sample contained all the reaction reagents except the extract. After 2 h of incubation at 25 °C, the absorbance was measured at 760 nm with a spectrophotometer (JENWAY 6300). Total phenols were expressed as gallic acid equivalents. The experiment was conducted in four replications.

Statistical analysis

The statistical analysis of data was carried out using Microsoft Excel. The mean values, standard deviations, and significant values were calculated. Statistical significance was set at $p < 0.05$.

Results and discussion

Grain viability is an important qualitative index. It characterizes grain capacity for germination maintenance of living grains shown in percentage [8, 9]. The obtained results show that only 92 ± 1 % barley grain "Class" was viable. Therefore, the determined grain sprouting activity was recalculated considering grain mean viability.

Copper is located in the very important parts of vegetable organisms, mainly in the growing points and germ. It increases the activity of phenol oxidase and ascorbic acid oxidase enzymes, influences plant carbohydrates and energetic metabolism, and promotes the formation of compounds with high energy phosphate bonds, providing energy for the fixation of molecular nitrogen N_2 [10, 11].

Copper as a trace element of a variable value is part of coenzymes participating in electron transfer enzyme systems related to N_2 fixation. Copper also participates in the formation reactions of protein and amino acids [10].

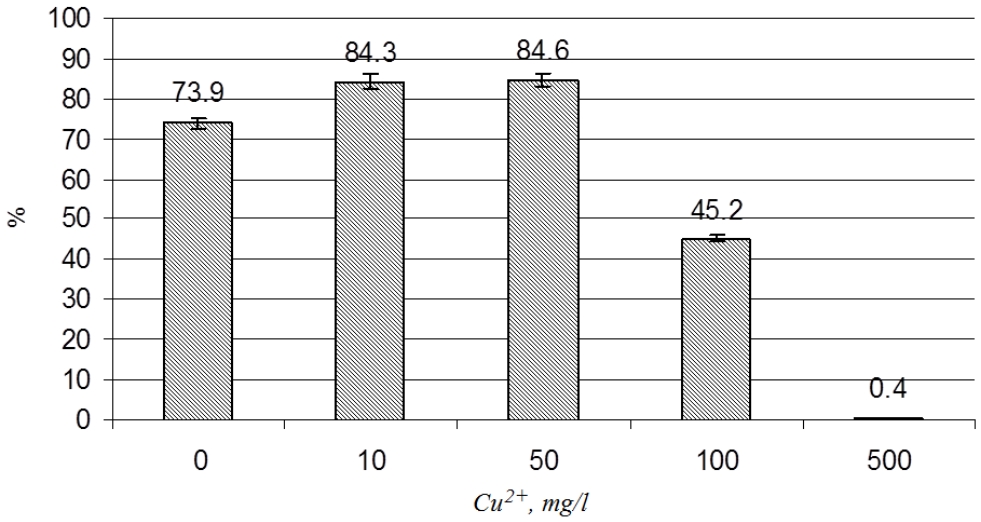

Fig. 1. The influence of different Cu^{2+} concentrations on barley grain sprouting considering grain viability

The obtained results (Fig. 1) showed barley grain sprouting activity to depend on copper concentration in the solution, but not all analyzed Cu^{2+} concentrations promoted the sprouting of barley grain.

It is well known that polyphenols present in beverages play an important role in their quality and stability as well as in the prevention of some pathologies [14].

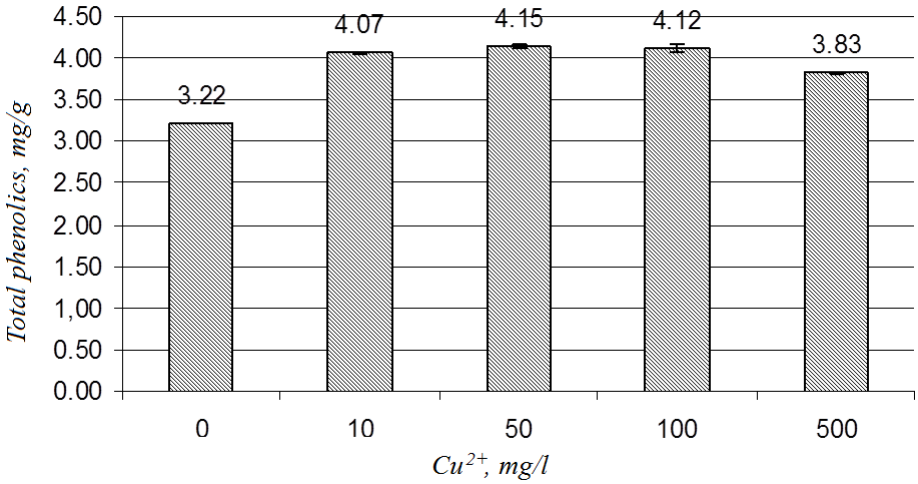

Fig. 2. The influence of different Cu^{2+} concentrations on the content of total phenols

The obtained results (Fig. 2) have shown that all analyzed copper concentrations in solutions influence the content of total phenols in germinated barley grains. The increase of total phenol content was significant ($p < 0.05$) at all analyzed copper concentrations, and the highest content (4.15 mg/g) was observed when the copper concentration in the solution was 50 mg/l.

Conclusions

Copper concentration below 50 mg/l in a solution promoted barley grain sprouting during germination by 14 % as compared with the control sample. With increasing copper concentration up to 100 mg/l, the sprouting activity decreased, but at the concentration of 500 mg/l all life processes in grain was suspended. Copper additives had a positive effect on the formation of total phenols at all concentrations analyzed in the present study.

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SKIRTINGOS KONCENTRACIJOS VARIO TIRPALŲ ĮTAKA MIEŽIŲ GRŪDŲ DAIGUMUI IR BENDRAJAM FENOLINIŲ JUNGINIŲ KIEKIUI JUOSE

S a n t r a u k a

Miežių grūdai 12 val. buvo mirkomi skirtingos koncentracijos vario tirpaluose (Cu^{2+} koncentracija 10–500 mg/l), po to 5 dienas paliekami 18 ± 2 °C temperatūroje daiginti ir 48 valandas džiovinami 50 °C temperatūroje. Juos mirkant 10–50 mg/l koncentracijos vario tirpaluose, reikšmingai padidėja miežių grūdų dygimo aktyvumas, tačiau koncentracijai padidėjus iki 100 mg/l, daigumas gerokai sumažėja, esant 500 mg/l koncentracijai, grūdai visai nedygsta.

Miežių grūdus mirkant vario tirpaluose, padidėja bendrasis fenolinių junginių miežių grūduose kiekis.