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DETERMINATION OF HEAVY METALS AND NUTRITION VALUES IN BROCCOLI

OZNACZANIE ZAWARTOŚCI METALI CIĘŻKICH I SKŁADNIKÓW ODŻYWCZYCH W BROKUŁACH

Abstract: The aim of this study was to determine the content of chosen heavy metals (mercury, lead and cadmium) and some nutritional substances (β-carotene and vitamin C) in raw broccoli samples obtained from trade network. For the assessment of heavy metals the samples were mineralized with the mixture of sulphuric acid and hydrogen peroxide and the analysis was performed using atomic absorption spectroscopy (analyzers AMA 254 and AVANTA GBC 933A, GBC, Australia). Vitamin C and β-carotene were extracted by hexane and acetone and mixture of methanol, phosphoric acid and redistilled water, respectively. Both β-carotene and vitamin C were determined by high-performance liquid chromatography with electrochemical detection (Coulochem III, ESA, USA). The broccoli samples contained 0.094 ± 0.115 μg · 100 g^{-1} of mercury and 0.0004 ± 0.0398 μg · 100 g^{-1} of lead. No cadmium was detected. It appears from this results that no heavy metals accumulate in this vegetable since all concentrations were below quality standard. The amount of β-carotene in broccoli was 1.703 ± 0.194 mg · 100 g^{-1} and the content of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1}.

Keywords: broccoli, HPLC, Coulochem III, Pb, Cd, Hg, vitamin C, β-carotene, AAS

Diets rich in fruits and vegetables are protective against disease and populations that consume such diets have higher plasma antioxidant status and exhibit lower risk of cancer and cardiovascular disease [1]. Whether the health benefits of antioxidant-rich diets are due wholly or in part to their antioxidant capacity is controversial, but increased uptake of antioxidants from food is promoted globally as a simple and potentially highly effective means of health promotion [2]. There are many different kinds of antioxidants and nutrients in foods, and it is impossible to measure all [3].

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Members of the genus *Brassica* belong to the Cruciferous family and are reported to posses both antioxidant and health-promoting properties [4]. Cruciferous vegetables, such as broccoli can be eaten uncooked, but are most commonly eaten after cooking by steaming, boiling or microwaving. Broccoli is marketed as either a fresh or a processed product (eg, frozen or chopped). The United States per capita consumption of fresh broccoli has steadily increased over the last two decades [5], mainly because of its popularity in salad bars. In addition, broccoli has high vitamin C, vitamin A, fiber and mineral content, saccharides, folates, plus several cancer-preventing agents, which make broccoli a popular item among health-conscious consumers [6]. But, sometimes it is important to control heavy metals in broccoli. In addition, fresh broccoli is highly perishable, with a shelf-life of 3–4 weeks in air at 0 ^{o}C, up to 2 weeks at 4 ^{o}C, but only 2–3 days when kept at room temperature [7], mostly due to the relatively high rate of metabolism and consequent high respiration rate [8].

Materials and methods

The concentrations of three heavy metals, namely, Cd, Pb, and Hg, in the broccoli were determined by the AAS method, using the analyzers AMA 254 and AVANTA GBC 933A. Liquid sample was normally turned into an atomic gas, atomization was made up with acetylene. Samples of broccoli were mineralized with H_2SO_4 and H_2O_2. Results were evaluated on the basis of measuring calibration curves.

Broccoli has a very powerful nutritional profile, containing loads of vitamins and minerals such as vitamin A, thiamin, riboflavin, niacin, vitamin C, vitamin E, folate, vitamin K, pantothenic acid, calcium, copper, iron, magnesium, phosphorus, potassium, zinc and etc. Many authors reported that the raw green broccoli contains per 100 g edible portion (tough stems removed, 61 % of product as purchased): water 88.2 g, energy 138 kJ (33 kcal), protein 4.4 g, fat 0.9 g, carbohydrate 1.8 g, dietary fibre 2.6 g, carotene 575 μg, thiamin 0.10 mg, riboflavin 0.06 mg, niacin 0.9 mg, folate 90 μg, ascorbic acid 87 mg etc. It follows broccoli contains many antioxidants, including carotenoids, tocopherols, ascorbic acid, and flavonoids [9, 10]. Due to this we were interested in amount of β-carotene and vitamin C, especially. In our study HPLC system ESA equipped with Coulochem III detector was used. In the case of β-carotene broccoli sample was extracted by mixture of acetone:hexane (1:1, v/v) several times in water bath 27 ^{o}C, samples were protected from sun light, of course. After its at solvents were removed using rotary vacuum evaporator. Rest of sample was diluted into HPLC quality ethanol and injected into the column. Measurement conditions were following: inject volume 20 mm^3, column Supelcosil LC-8 (150 × 4.6 mm, 5 μm), temperature 30 ^{o}C, mobile phase $CH_3OH : H_2O : H_3PO_4$ (99 : 0.5 : 0.5, v/v/v), flow rate 1.1 cm$^3 \cdot$ min^{-1} in isocratic elution, detector was set to 300 and 400 mV, guard cell 750 mV. Under the same condition, calibration curve was measured. In the case of vitamin C determination sample was extracted by mobile phase directly for a period of 15 min in shaker bath at 25 ^{o}C. Sample was protected from sun light during all extracted process. After that, sample was filtered and injected into the HPLC system. Measurement conditions were following: injected volume 20 mm^3, column Supelcosil LC-8 (150 × 4.6 mm, 5 μm), temperature 30 °C, mobile phase $CH_3OH : H_2O : H_3PO_4$ (99 : 0.5 : 0.5, v/v/v), flow rate 1.1 $cm^3 \cdot min^{-1}$ in isocratic elution, detector was set to 600 and 650 mV, guard cell 750 mV. Calibration curve was measured under the same condition.

Results and discussion

Lead is a poisonous metal that can damage nervous connections and cause blood and brain disorders. Long term exposure to lead or its salts can cause nephropathy and colic-like abdominal pains. Mercury is a cumulative heavy metal poison, all mercury--based toxic compounds damage the central nervous system and other organs or organ system such as the liver or gastrointestinal tract. Cadmium is one of the substances banned by the European Union's Restriction on Hazardous Substances, including proteinuria and glucosuria, cadmium-containing compounds are known carcinogens and can induce many types of cancer. We found out that our tested broccoli samples obtained 0.094 ± 0.115 $\mu g \cdot 100\ g^{-1}$ of Hg, 0.0004 ± 0.0398 $\mu g \cdot 100\ g^{-1}$ of Pb. We determined no cadmium. It appears from this that no heavy metals accumulate in this vegetable, all results were below the quality level. The level of significance was set at 95 %.

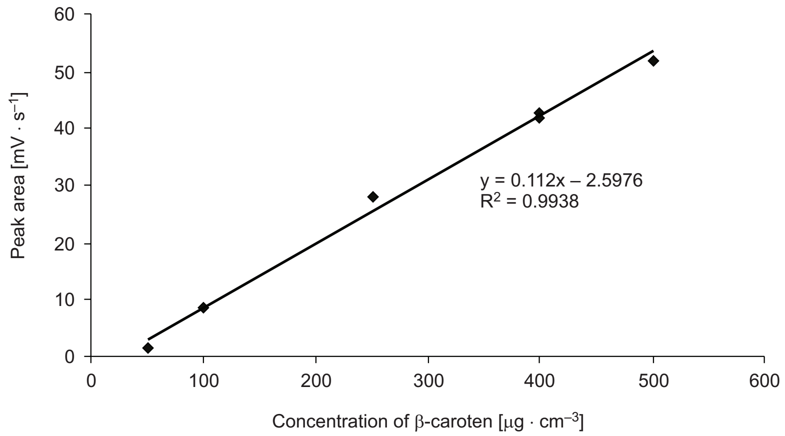

Fig. 1. Calibration curve of β-carotene

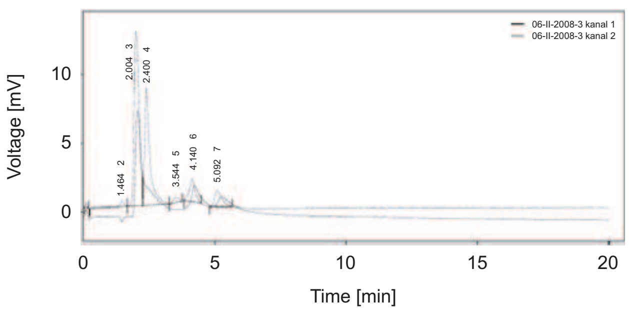

Fig. 2. Chromatogram: β-carotene in raw broccoli

β-Carotene is the most effective vitamin A precursor, and has been reported to protect humans against certain types of cancer and cardiovascular diseases [10,11]. Calibration curve was obtained analyzing five different solution of known concentration of β-carotene standard included between 50–500 μg · cm^{-3}. The curve equation y = ax + b calculated with linear regression method was utilized to determine samples concentration. Calibration curve is shown in Fig. 1. The results show that β-carotene can be determined in broccoli by HPLC with ECD, β-carotene retention time was 3.6 min. The result of our experiment showed that amount of β-carotene was 1.703 ± 0.194 mg · 100 g^{-1} in raw broccoli. The literature β-carotene content determined in leafy vegetables is 0.5–2 mg · 100 g^{-1} [5, 10, 11].

Vitamin C is considered to be the most important vitamin for human nutrition which could be best supplied by fruits (especially citrus and some tropical fruit) and vegetables. Standard solutions of L-ascorbic acid were prepared in a solution corresponding to the mobile phase (calibration curves of concentration 1–4 $mm^3 \cdot cm^{-3}$) used in isocratic conditions. Triplicate injections for each standard solution were made and the peak area was plotted against the corresponding analyte concentration to obtain the calibration curves y = 72.651x – 46.433. Results are presented in Fig. 3 and 4. Retention time of vitamin C was 1.8 min.

We determined that amount of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1} in raw material. There are lots of articles describing concentration of vitamin C in broccoli, average value ranges from 20 to 90 mg · 100 g^{-1}[4, 5, 7, 8]

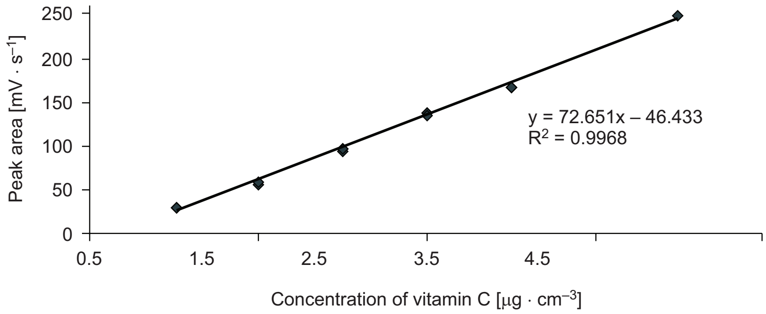

Fig. 3. Calibration curve of vitamin C

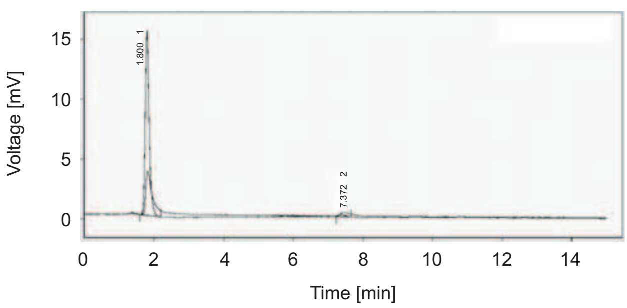

Fig. 4 Chromatogram: Vitamin C in raw broccoli

Conclusions

For determination of mercury (Hg), cadmium (Cd) and lead (Pb) analyzers AMA 254 and AVANTA GBC 933A were used. We found out that our tested broccoli samples contained 0.094 ± 0.1152 μg · 100 g^{-1} of Hg, 0.0004 ± 0.03981 μg · 100 g^{-1} of Pb. We determined no cadmium. The ECD ability to measure low levels of vitamin and flavonoids and carotenoids can provide a competitive advantage by profiling the characteristic qualities of products, to their commercial values. The result of our experiment showed that amount of β-carotene was 1.703 ± 0.1945 mg · 100 g^{-1} in raw broccoli sample and amount of vitamin C was 57.974 ± 0.535 mg · 100 g^{-1} in raw material. A high intake of broccoli has been found to reduce the risk of aggressive prostate cancer. Broccoli leaf is also edible and contains far more β-carotene than the florets.

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OZNACZANIE ZAWARTOŚCI METALI CIĘŻKICH I SKŁADNIKÓW ODŻYWCZYCH W BROKUŁACH

Abstrakt: Celem badań było zbadanie zawartości wybranych metali ciężkich (rtęć, ołów i kadm) oraz niektórych składników odżywczych (β-karoten i witamina C) w świeżych brokułach. W celu oznaczenia zawartości metali ciężkich próbki były mineralizowane w mieszaninie kwasu siarkowego i nadtlenku wodoru. Oznaczenia wykonano metodą spektrofotometrii absorpcji atomowej (analizatory AMA 254 and AVANTA

GBC 933A, GBC, Australia). Witaminę C i β-karoten izolowano przy użyciu kolejno heksanu i acetonu oraz mieszaniny metanolu, kwasu fosforowego i wody destylowanej. Witaminę C i β-karoten oznaczono przy użyciu chromatografii cieczowej oraz analizy elektrochemicznej (Coulochem III, ESA, USA). Próbki brokułów zawierały 0.094 ± 0.115 μg · 100 g^{-1} rtęci oraz 0.0004 ± 0.0398 μg · 100 g^{-1} ołowiu. Kadm nie został wykryty. Przeprowadzone badania wydają się wskazywać, że brokuły nie kumulują metali ciężkich. β-karoten występował w brokułach w ilości 1.703 ± 0.194 mg · 100 g^{-1}, a zawartość witaminy C wynosiła 57.974 ± 0.535 mg · 100 g^{-1}.

Słowa kluczowe: brokuł, HPLC, Coulochem III, Pb, Cd, Hg, witamina C, β-karoten