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SCREENING OF SELEMIUM CONTENT OF SOME SPICES AND WHEAT PRODUCTS AND STUDYING OF ITS CYTOTOXIC EFFECT ON HUMAN TUMORS.

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ABSTRACT

Screening the amount of selenium in some spices and wheat products was carried out. Among 20 samples, anise had the highest amount of selenium (0.687 ppm). For wheat products, wheat bran contained the highest level of selenium (0.363 ppm). The optimal pH for extracting selenium from wheat bran was 6. Selenium showed a cytotoxic activity against brain tumor cell line (U251), human hepato cellular cell line (Hepg2) and lung carcinoma cell line (H460).

INTRODUCTION

Thousands of chemical structures have been identified in plant foods. Many are found in spices. Typically, spices are the dried aromatic parts of plants (generally the seeds, berries, roots, pods, and sometimes leaves) that mainly, but not invariably, grow in hot countries. Given the wide range of botanical species and plant parts from which spices are derived, they can contribute significant variety and complexity to the human diet. In the past, the medicinal uses of spices and herbs were often indistinguishable from their culinary uses, and for good reason: people have recognized for centuries both the inherent value, as well as the potential toxicity, of phytochemicals in relation to human health (Lampe, 2003). The effects of selenium and numerous phytochemicals in spices and herbs are covered by experts in the field. Research on selenium indicating its effect on cancer prevention at doses higher than those required for normalizing body selenium stores is of particular importance in setting the future research agenda on phytochemicals. The hypothesis that organoselenium metabolites can penetrate avascular tumor tissue and inhibit angiogenesis serves as an important potential research direction for studies of many different phytochemicals found in fruit and vegetables (Heber, 2002).

There are several selenocompounds in tissues of plants and animals. Selenate is the major inorganic selenocompound found in both animal and plant tissues. Selenocysteine is the predominant selenoamino acid in tissues when inorganic selenium is given to animals. Selenomethionine is the major selenocompound found initially in animals given this selenoamino acid, but is converted with time afterwards to selenocysteine. Selenomethionine is the major selenocompound in cereal grains, grassland legumes and soybeans. Selenomethionine can also be the major selenocompound in selenium enriched yeast, but the amount can vary markedly depending upon the growth conditions. Se-methylselenocysteine is the major selenocompound in

selenium enriched plants such as garlic, onions, broccoli florets and sprouts, and wild leeks (Whanger, 2002). It is widely distributed throughout the body, but is particularly well maintained in the brain, even upon prolonged dietary selenium deficiency (Chen and Berry, 2003).

The nutritional essentiality of selenium was first suggested in 1957, when Schwarz and Foltz reported that dietary intake of this trace element protected against necrotic liver degeneration in rats fed vitamin E-deficient diet. Moreover, selenium has been intensively investigated as an antioxidant trace element. It is also an integral component of the enzyme glutathione peroxidase and other selenoenzymes or selenoproteins which are involved in the removal of hydrogen peroxide and lipid peroxides produced during oxidative process in the cells. This function helps to maintain membrane integral and likelihood of propagation of further oxidative damage to lipids, lipoprotein and DNA with the associated increased risk of conditions such as atherosclerosis (Rotruck *et al.*, 1973 and Ne`ve, 1996). Selenium has several different tasks in the body such as preventing the rancidification of the cells and cell damage which delays the pathological ageing process, preventing the blood clots by inhibiting platelet aggregation, increasing the effectiveness of the immune system, strengthening the resistance to viral and bacterial infection and counteracting adverse effects of heavy metals and other toxic substance in the body (Tolenone, 1990).

Selenium deficiency in humans may cause some diseases such as cancer (Pike and Brown, 1975), cataracts disease (Fecondo and Augusteyn, 1983), ischemic heart disease (Ellis *et al.*, 1984), Sclerosis disease (Jensen and Clausen, 1984), rheumatoid arthritis (Trap *et al.*, 1985), muscular dystrophy disease (Omdahl *et al.*, 1986), and Keshan disease (Cases *et al.*, 2002).

Selenium would appear to prevent cancer in at least four different ways: it protects the cells from damage caused by oxygen free radicals, it decreases the mutagenenesis of carcinogens, it inhibits the reproduction of carcinogenic viruses, and it inhibits the division of cancer cells (Tolenen, 1990). Some studies indicated that mortality from cancer, including lung, colorectal, liver, brain, prostate and nonmelanoma skin, is lower among people with higher selenium blood levels or intake (Knekt *et al.*,1998 and Young & Lee, 1999). Changes in selenium concentration in blood and brain have been reported in Alzheimer's disease and brain tumors (Chen and Berry, 2003).

The purpose of this study was to investigate the hypothesis that selenium has an inhibiting effect against cancer. Therefore, some spices and some wheat products with added spices were collected from Egyptian local market and examined for their selenium content. The potential cytotoxic activity of selenium against human tumor cell lines was examined for brain, breast, liver and lung tumors.

MATERIALS AND METHODS

Spices (anise, sesame, turmeric, mahaleb, black seed, thyme, marjoram, bay leafs, cumin, safflower, carnation, mesteka, coriander, fennel, rosemary, black pepper, cardamom, nutmeg, capsicum, and cubeb), raw materials, and bakery products were purchased from the local market.

Moisture content of all samples was determined according to the method described in A.O.A.C. (1990).

Determination of selenium in all samples was carried out according to the method described in A.O.A.C. (1990) as follow: 1-One gram sample (dry matter) was placed into 100 ml Kjeldahle flask containing 10 ml H_2O and swirled to wet sample. 2-Ten ml HNO_3 were added and heated continuously to reduce volume to about 5 ml, then cooled. 3- Six ml $HClO_4$ and 5.0 ml H_2SO_4 were added and reheated until turned to yellow, and then became colorless. 4- After cooling the solution was made up to 25 ml with deionized water and read using Perkin- Elmer hydride generation atomic absorption spectrophotometer.

To determine the optimal pH for selenium extraction from wheat bran, different bran pH-solutions (2, 4, 8, 7, 8, 10, 12, and 14) were prepared using citric acid and sodium hydroxide. Each bran solution was homogenized (Tempest Virtishear homogenizer) at 10000 r.p.m. for 10 min. The mixture was filtrated through ashless filter paper No.42 then the volume was made up to 25 ml with deionized water and selenium was measured as previously mentioned.

The cytotoxicity of selenium against human tumor cell lines was conducted at the National Cancer Institute, Cairo University, Egypt. Four cell lines were used in the present work : brain tumor cell line (U251), breast carcinoma cell line (MCF7), human hepato cellular cell line (Hepg2) and lung carcinoma cell line (H460).The experiment was carried out as described by Skehan *et al.* (1990). The cytotoxicity was measured by Sulforhodamine B stain (SRB) assay. Cells were plated in 96-multiwell plate for 24 hr before treatment with the sodium selenite (Acros Organics Com.) solution to allow attachment of cell to the wall of the plate. Different concentrations of selenium solution (1-10µg/ml) were added to the cell monolayer and then incubated at 37° C, 5% CO_2 for 48 hr. After 48 hr cells were fixed, washed, and stained with SRB. Color intensity was measured in an ELISA reader. The relation between surviving fraction and selenium concentrations was plotted to get the survival curve of each tumor cell line. The parameters used for the evaluation of the cytotoxic activity of selenium against human tumor cell lines were the 50% growth inhibition (GI_{50}) and 10% growth inhibition (GI_{10}).

RESULTS AND DISCUSSION

Selenium content of different spices and raw materials are shown in Table (1). It could be noticed from the table that the highest three samples of selenium content were first anise (0.687 ppm) followed by safflower (0.543

ppm), then sesame (0.471 ppm). On the other hand, the lowest three samples of selenium amount were found in mesteka (0.133 ppm), coriander (0.155 ppm), then nutmeg (0.183 ppm). While the selenium concentration of the other samples ranged between the two previous parameters. These results are in agreement with those reported by Mayland *et al.* (1989) who stated that concentration of selenium in most plants depends on the amount and type of selenium in the soil, irrigated water, differences in rooting depth and genetic traits that affect the absorption and translocation of selenium to shoot.

Table (1): Selenium content of some spices Sample

Sample	Selenium content (ppm)
Anise	0.687
Sesame	0.471
Turmeric	0.369
Mahaleb	0.349
Black seed	0.252
Thyme	0.408
Marjoram	0.355
Bay leafs	0.244
Cumin	0.198
Safflower	0.543
Carnation	0.228
Fennel	0.221
Mesteka	0.133
Coriander	0.155
Rosemary	0.257
Black pepper	0.316
Cardamom	0.297
Nutmeg	0.183
Capsicum	0.327
Cubeb	0.246

Data in Table (2) illustrate the selenium concentration of some wheat products supplemented with spices. Therefore, samples such as zwieback, anise-zwieback, sesame-zwieback, turmeric-zwieback, mahaleb-zwieback, black seed-zwieback, flour (72% ext.), bran, rolls, white bread (72% ext.), bread (82% ext.), and bran bread were collected from Egyptian local market. From the table it could be observed that supplementation of zwieback with anise, sesame, turmeric, mahaleb, and black seed increased the selenium concentration from 0.217 ppm (control), to 0.297, 0.406, 0.283, 0.284, and 0.221 ppm, respectively. This increasing might be due to the selenium content in the supplementing materials taking into consideration that these materials were not added in a fixed percentage. Also, it could be seen from the same table that comparing the other wheat products to each other resulted in concluding that wheat bran had the highest amount of selenium (0.363 ppm) followed by bran bread, bread (82% ext.), rolls and white bread (72% ext.) with selenium amount of 0.278, 0.234, 0.218 and 0.220 ppm, respectively. Subsequently, it could be concluded that the higher percentage of bran led to the higher content of selenium in the produced product.

Table (2): Selenium content of some wheat products Sample

Sample	Selenium content (ppm)
Zwieback	0.217
Anise-zwieback	0.297
Sesame-zwieback	0.406
Turmeric-zwieback	0.283
Mahaleb-zwieback	0.284
Black seed-zwieback	0.221
Flour (72% ext.)	0.213
Bran	0.363
Rolls	0.218
White bread (72% ext.)	0.220
Bread (82% ext.)	0.234
Bran bread	0.278

Since wheat bran contained the highest amount of selenium, it was chosen for further investigation to determine the optimal pH for selenium extraction. As it could be noticed from data in table (3) that the selenium content of wheat bran increased when the pH increased up to 6 as the amounts of selenium were 0.401, 0.458, and 0.501 ppm at pH values of 2, 4, and 6, respectively. Moreover, the same table shows that increasing the pH from 6 to 14 decreased the amount of selenium from 0.501 to 0.349 ppm. So, it could be concluded that the optimal pH for extraction selenium from wheat bran was at 6.

Table (3): Effect of pH on selenium content of wheat bran

Sampledifferent pH	Selenium content (ppm)
Bran (control)	0.363
Bran pH 2	0.401
Bran pH 4	0.458
Bran pH 6	0.501
Bran pH 7	0.497
Bran pH 8	0.395
Bran pH 10	0.371
Bran pH 12	0.357
Bran pH 14	0.349

The selenium solution was tested for any cytotoxic activity against human breast carcinoma cell line (MCH7), human hepato cellular carcinoma cell line (Hepg2), brain tumor cell line (U251), and lung carcinoma cell line (H460). The results are shown in Table (4) and fig. (1). It could be noticed that selenium was proven to have cytotoxic activity against human tumor cell lines. Selenium reduced the survival of human brain tumor cell line (U251) to 50% at concentration of 0.5µg/ml. The same trend was seen for the human hepato cellular carcinoma cell line (Hepg2) as selenium reduced the survival to 50% at concentration of 0.5µg/ml. The same table showed that the survival of lung carcinoma cell line (H460) was reduced to 50% at concentration of 6.35µg/ml.On the other hand, selenium effect on human breast carcinoma cell line to 50% was not detected. In this concern, the anticarcinogenesis effect of selenium was explained by Chen and Berry (2003) who reported that the functions of selenium are believed to be carried out by selenoproteins, in which selenium is specifically incorporated as the amino acid, selenocysteine. Glutathione peroxidase has been localized in glial cells, and its expression is increased surrounding the damaged area in Parkinson's disease and occlusive cerebrovascular disease, consistent with its protective role against oxidative damage. Selenoprotein P has been reported to possess antioxidant activities and the ability to promote neuronal cell survival. Recent studies in cell culture and gene knockout models support a function for selenoprotein P in delivery of selenium to the brain mRNAs for other selenoproteins, including selenoprotein W, thioredoxin reductases, 15-kDa selenoprotein and type 2 iodothyronine deiodinase, are also detected in the brain. In addition, Santamaría *et al.* (2003) stated that quinolinic acid (QUIN), a well known excitotoxin that produces a pharmacological model of Huntington's disease in rats and primates, has been shown to evoke degenerative events in nerve tissue via *N* -methyl-d-aspartate receptor (NMDAr) overactivation and oxidative stress. It was found that the antioxidant selenium (as sodium selenite) partially protects against QUIN toxicity.

Table (4): The cytotoxic activity of selenium against human brain tumor cell line (U251), breast tumor cell line (MCF7), liver tumor cell line (Hepg2) , and lung tumor cell line (H460).

Cell line	IC_{50}	IC_{10}
U251 (brain)	0.5 µg/ml	Nd
MCF7 (breast)	Nd	Nd
Hepg2 (liver)	0.5 µg/ml	Nd
H460 (lung)	6.35 µg/ml	Nd

IC_{50} : Dose of selenium which reduces survival to 50%
IC_{10} : Dose of selenium which reduces survival to 10%
Nd : Not detected

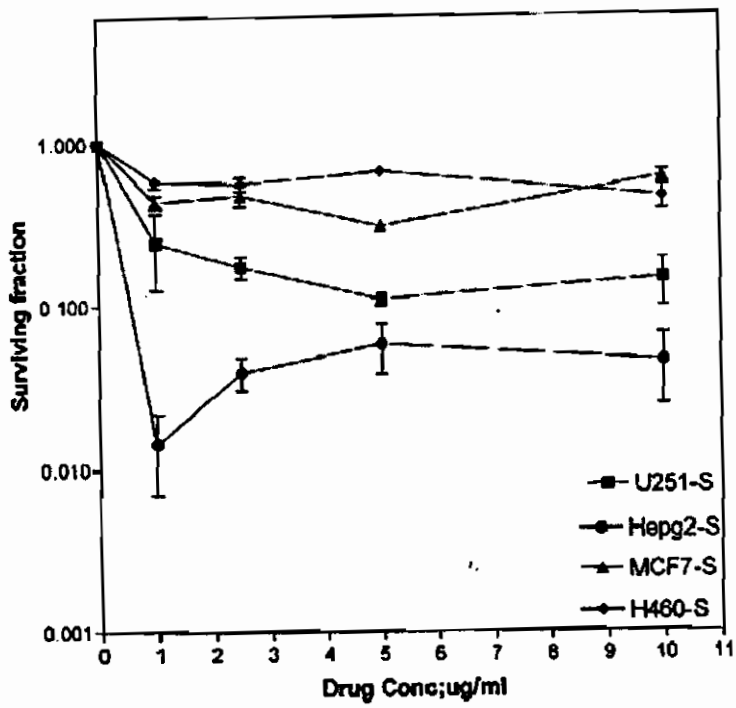

Fig. 1: The cytotoxic activity of selenium against human breast carcinoma cell line (MCH7), human hepato cellular carcinoma cell line (Hepg2), human brain tumor cell line(U251), and lung tumor cell line (H460).

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مسح لمحتوى السلينيوم فى بعض التوابل ومنتجات القمح ودراسة تأثيره السام للخلايا السرطانية فى الإنسان

بدوية حمزة - هانئ عبد العزيز فهمى
معهد بحوث تكنولوجيا الأغذية - مركز البحوث الزراعية - الجيزة - مصر

تم تقدير عنصر السلينيوم فى عدد من العينات المسحوبة من السوق المحلى مثل الينسون - السمسم - الكركم - المحلب - حبة البركة - الزعتر - البردقوش - ورق اللورى - الكمون القرطم - القرنفل - المستكة - الكزبرة - الحلبة - حصى ألبان - الفلفل الأسود - الحبهان - جوزة الطيب - الشطة - الكبابة - وأيضا لبعض منتجات القمح مثل البقصمات -والبقصمات بالينسون - البقصمات بالسمسم - البقصمات الكركم - البقصمات بالمحلب - البقصمات بحبة البركة - دقيق ٧٢% إستخلاص - الردة - الفينو - الخبز الأبيض - الخبز ٨٢% إستخلاص - خبز السن.

وأثبتت النتائج الآتى:

- يحتوى الينسون على أعلى نسبة من عنصر السلينيوم وهى ٠,٦٧٨ جزء فى المليون .
- تحتوى الردة على أعلى نسبة من عنصر السلينيوم وهى ٠,٣٦٣ جزء فى المليون .
- كانت درجة الحموضة المثلى لإستخلاص عنصر السلينيوم من الردة هى ٦.

-وقد أظهرت النتائج أيضا أن عنصر السلينيوم له تأثير مثبط لنمو خلايا سرطان المخ والكبد والرئة.