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Researching the Content of Chemopreventors in Plant Raw Materials

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Abstract

Functioning of the human organism requires a lot of minor compounds. With the development of science, the information about these substances expands. Most of them are difficult to make up through the diet, as they are contained in the chemical composition of natural raw materials, which people cannot consume all the time, or in large quantities. Such compounds include chemopreventors, which are present in the composition of plant raw materials. The authors of the article have suggested a weakly studied source of chemopreventors– *Raphanus sativus* (black radish). In studying the chemical composition of the object, refined data about the content of biologically active substances have been obtained. It has been found that the content of glucosinolates in radish black correlates with their contents in broccoli. During the heat treatment, the loss of chemopreventors does not exceed 26 %.

Keywords: study**,** chemopreventors, *Raphanus sativus* biologically active compounds, glucosinolates, isothiocyanates, indole compounds.

Introduction

Currently, based on the development of chemistry, biology, toxicology, and medicine, new data have been obtained about minor biologically active substances, which were not considered previously as factors necessary for human life. Participation of many of them in a number of metabolic processes has been proven. Such natural compounds, which are commonly referred to as chemopreventors, include phenolic compounds, dietary indoles and isothiocyanates, and many others. Despite the fact that the clinical picture of deficiency of these compounds has not been determined, their low concentration in the daily diet of humans is accompanied by a significant increase in the risk of development of many common diseases [1].

Phenolic compounds have a specific biological effect on various functions of individual metabolic systems and the entire organism: immunomodulatory, antitumoral, antiviral, antimutagenic, adaptogenic, etc. The most important manifestation of physiological and pharmacological action of the phenolic compounds is their P-vitamin action manifested in decreasing the permeability and increasing the strength of capillary walls. Many substances with P-vitamin action are glycosides of flavonols and flavanones, or aglycones [2].

Natural chemopreventors are products of glucosinolates hydrolysis — dietary indoles and isothiocyanates. Glucosinolates activate the enzymes that neutralize xenobiotics, inhibit the tumor growth factors, prevent the formation of new blood vessels in tumors, inhibit division, induce apoptosis of tumor cells, and have an antioxidant effect [3, 4].

By their chemical nature, glucosinolates are glycosides, the molecules of which consist of carbohydrate portions and aglycones, bound via atoms of carbon, oxygen, sulfur, or nitrogen. Glycosides differ from each other by the aglycone and the carbohydrate chain structure. Aglycones are unstable compounds capable of spontaneous rearrangement with the formation of several products, depending on the structure of the side chain and the conditions of the reaction. Variables side chains may be represented by methionine, tryptophan, phenylalanine, or other amino acids with branched chains.

Glucosinolates contained in vegetables [5] are chemically and thermally stable, but if their cell compartment is damaged, their hydrolysis occurs under the action of the myrosinase enzyme, accompanied by the formation of stable and unstable isothiocyanates.

In the neutral medium, the main products of glucosinolates hydrolysis are stable isothiocyanates R-N=C=S, except for those that contain an indole group. Unstable β-OH-isothiocyanates are subjected to cyclization with the formation of oxazolidin-2-thions (e.g., goitrin), and indole isothiocyanates convert into their corresponding alcohols, for example, indole-3-carbinol [6].

Indoles increase the biotransformation activity of cells over 50 times. They have hepatoprotective and hepatoinducingproperties; prevent pathological changes of the estrogen that is carcinogenic to the gastrointestinal tract, pancreas and thyroid glands, and the reproductive organs in males and females [7-9]. Dietary indoles can significantly change the activity and the ratio of enzymes, including various isoforms of the P-450 cytochrome, which are involved in the metabolism of various xenobiotics and many endobiotics. They also can reduce the risk of certain types of hormone-dependent tumors. In particular, indole-3-carbinol inhibits carcinogenesis of the mammary glands, cervix and uterine body, prostate, colon, tongue, liver, lungs, and skin [10].

Like indoles, isothiocyanates have anti-carcinogenic properties. They are strong inducers of xenobiotics metabolism phase II enzymes. Unlike indoles, they do not affect the activity of monooxygenases but can inhibit some isoforms of the P-450 cytochrome, engaged in the activation of benzo(a)pyrene, aflatoxin B1, and nitrosamines by means of competitive inhibition or covalent inactivation. Some authors have found in course of *in vivo* experiments that isothiocyanates can inhibit carcinogenesis of the colon, esophagus, pancreas, oral cavity, lungs, mammal glands, and bladder [11-13].

Currently, sources of chemopreventors are biologically active additives made by various companies, which include indole-3-carbinol isolated from plant materials or synthesized, with the addition of powder broccoli concentrate as a source isothiocyanates. The content of indole-3-carbinol varies from 90 to 200 mg in a single capsule.

Biologically active additives (BAA) are not medications and are therefore recommended for long time administration. However, all BAA are capsules, which are in turn associated with medications. BAA with indole-3-carbinol powder include broccoli powder since the efficacy of the additive depends on the presence of both indole-3-carbinol and isothiocyanates. From the prevention point of view, BAAs are recommended for all people, especially for those who do not consume enough vegetables.

The main food sources of glucosinolates are cruciferous vegetables (all kinds of cabbage, garden cress, radish, and turnip). Works of a number of scientists have proven that consumption of cruciferous plants and the frequency of tumors' occurrence in the small intestine and colon, and estrogen-dependent tumors of the breast (in women) and prostate in men are closely inverse-related. This regularity has also been confirmed in working with individual substances and extracts from these plants, especially broccoli [14, 15]. The content of glucosinolates in cruciferous plants ranges from 50 to 390 mg/100 g of the product.

Of the cruciferous vegetables (*Cruciferae*), radish black (*Raphanus Sativus*) is most known for its healing and preventive properties. For over 4,000 years, it has been used as a medicinal herb; it has been eaten since the IV century A.D. Nevertheless, radish has been studied worse than broccoli and Brussels sprouts in terms of chemopreventors content. No accurate literature data about the content of glucosinolates in radish has been found.

This work was aimed at studying *Raphanus Sativus* as a source of glucosinolates.

MATERIALS AND METHODS

Raphanus sativus —radish black was used as the object of the research. In parallel, check studies were made on samples of broccoli. According to various literature sources, the content of isothiocyanates in it is (50 – 250) mg%, of indole compounds — (30 – 55) mg%.

Detection of glucosinolates was performed in three stages: identification by the characteristic analytic reactions; spectroscopy of functional groups in the infrared region; quantification.

For the purpose of indole compounds identification, analytical reactions of Wagner, Bouchard, Maillard, Sonnenstein, Scheibler, and Ehrlich were used. Quantification was based on the method based on extracting indole compounds with ether-chlorine mixture followed by titration.

The content of isothiocyanates was determined by stripping the extract with subsequent titration of thiourea derivative in an acidic medium.

In determining the sum of phenolic compounds, the method of redox titration was used. Potassium permanganate was used as the standard solution. The reaction was performed in an acidic medium.

The content of water-soluble vitamins was determined using the fluorometric method based on consequent acid and enzymic hydrolysis of the bound forms of vitamins, followed by subsequent measurement of the fluorescence intensity.

The content of minerals was determined by the method of X-ray fluorescence, which is based on the dependence of X-ray fluorescence intensity on the concentration of the element in the sample, with the use of spectrometer S4 Pioneer (Bruker AXS).

RESULTS AND DISCUSSION

Literature sources contain the data about the macronutrient composition of radish, without specifying a particular species. Moreover, the data about the content of biologically active substances are rare, they are basically limited to the listing of the components present without quantitative values. As a result of the research, it has been found that chemical composition of black radish is mainly represented by carbohydrates: mono and disaccharides (6.00 ± 0.32) %, fiber (2.98 ± 0.31) %, and pectin (0.80 ± 0.05) %. The soluble to insoluble form share was 1:2.6, respectively.

In the mineral composition, potassium prevailed over other elements and was equal to (365.16 ± 6.24) mg%. Compared to other representatives of the cruciferous family, in black radish, the potassium content is 1.5 times higher than in turnip, 1.2 times higher than in cabbage, and 1.4 times higher than in radish. The content of potassium in broccoli is 315 mg%, which is slightly lower than in radish black.

The vitamin composition is mainly represented by water-soluble vitamins. Of these, ascorbic acid dominates, compared to other vitamins.

Plant raw materials contain biologically active substances that reduce or prevent the formation of free radicals, i.e. have antioxidant properties. First and foremost, these are phenolic compounds, i.e. aromatic substances, which, in terms of their diversity, dominate plant foodstuffs. It has been found that the content of phenolic compounds in black radish is (3.74 ± 0.13) mg%. However, even with such concentrations of phenolic compounds, black radish has antioxidant properties and can inhibit adrenaline oxidation like a 5% ascorbic acid solution.

To establish the functional groups characteristic of the glucosinolates and phenolic compounds, the test samples were studied in the infrared region. In studying the spectra, bands in the range of (1,230 – 1,140) cm^{-1} were established, which corresponded to phenolic compounds. In the region of wavelengths equal to (1,500 – 1,400) cm^{-1}, peaks were found, which corresponded to functional groups (-N=C=S) of stable isothiocyanates.

The results of quantitative analysis have confirmed the data of spectral studies, and have proven the fact that the content of isothiocyanates and indole compounds in radish black is not inferior to that in broccoli (Fig. 1).

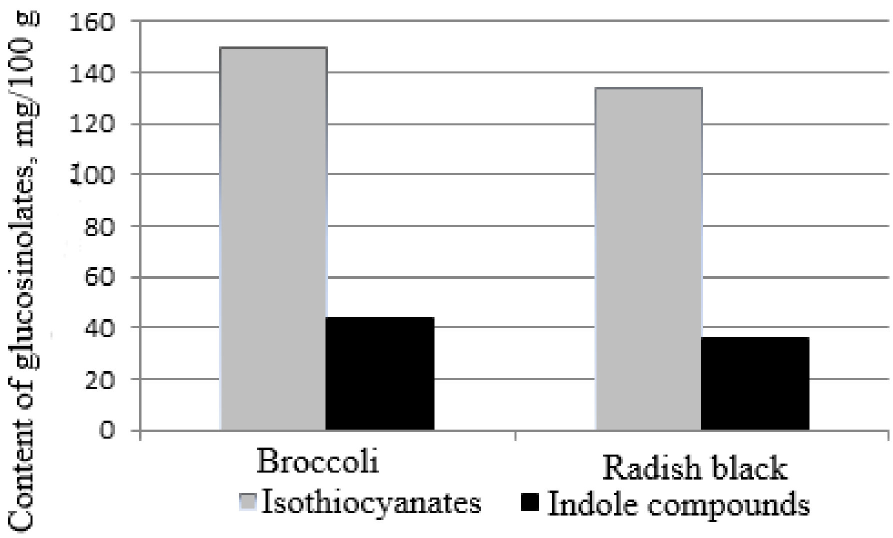

Figure 1 – The content of isothiocyanates and indole compoundsin the samples studied

Table 1. The content of glucosinolates after vegetables' heat treatment

Name	Black radish		Broccoli	
	(98 ± 2) °C	(220 ± 2) °C	(97 ± 2) °C	(220 ± 2) °C
Indole compounds, mg%	84.9 ± 2.2	78.7 ± 3.5	70.3 ± 2.5	63.8 ± 4.0
Isothiocyanates, mg%	83.6 ± 2.7	77.9 ± 3.8	69.2 ± 3.1	58.6 ± 4.2

The biological activity of glucosinolates depends not only on their presence but also on the indole compounds to isothiocyanates ratio. Thus, in broccoli, this ratio of 1:3.5 is considered to be optimal. As a result of studying the experimental samples, it has been found that the ratio of these compounds in radish black is comparable to that in broccoli, and is 1:3.6.

Preservation of biologically active substances by heat treatment is of practical interest. The temperature profiles generally adopted in the food industry were studied. The results of studying the loss of glucosinolates after heat treatment processing of radish black and broccoli are presented in Table 1.

It has been found that heat treatment of all experimental samples results in a decreased content of biologically active compounds. In samples of radish black, at the temperature of (98 ± 2) °C, the loss of indole compounds on the average was 15 %, and that of isothiocyanates — 16%. The "rougher" heat treatment (at the temperature of (220±2)^{o}C for 10 min) resulted in a decreased content of biologically active substances: to 24 % of indole compounds, and to 26 % of isothiocyanates. The decrease in the amount of glucosinolates in broccoli exceeded the same in radish black. Thus, at the temperature of (98±2) °C, the amount of biologically active substances decreased on the average by 30 %, at the temperature of (220±2) ^{o}C — by 42%, which was almost 2 times higher than in radish black. Therefore, heat treatment of radish black in various heat profiles results in losing chemopreventors by not more than 26 %. High losses in the samples of broccoli are perhaps related to the fact that broccoli is sold frozen, and used for food after defrosting and heat treatment.

CONCLUSIONS

Thus, it has been found that radish black by the content of glucosinolates is not inferior to broccoli, and can be used as a source of chemopreventors.

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