ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Technol. Aliment. 7(1) 2008, 5-17*

THE SOURCES OF NATURAL ANTIOXIDANTS

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Abstract. Intensive oxidative processes occuring in human organism lead to formation of oxygen reactive forms, which can damage systemic cells and tissues. It is shown, that body endogenous protective system can be supported in that case by natural antioxidant compounds provided from food. The assessment of food products as the potential sources of antioxidants was performed, taking into consideration the kinds of compounds supplied, and their significance in the diet of different nations.

Key words: antioxidants, polyphenols, source of antioxidants in diet

THE KINDS OF NATURAL ANTIOXIDANTS IN DIET

The results of epidemiological and scientific studies show that nutritional factor plays important role in prevention of the consequences of free radicals activity in the organism. A diet rich in natural antioxidants can significantly influence the increase of reactive antioxidant potential of the organism, and *ipso facto* decrease the risk of some diseases of free radicals origin. It is considered that adequate level of antioxidants supplied with diet induces immunological processes and increases defensive abilities of cell in proper way [Ghiselli et al. 2000, Prior 2003, Gałek and Targoński 2003, Grajek 2004].

There are mentioned: tocopherols (vitamin E), vitamin C, vitamin A, and also its provitamin β-carotene as well as selenium and phenolic compounds among antioxidants.

Properties and significance of vitamins A, C and E are quite well known being a subject (as a nutritional compound) of numerous studies for a long time [Wartanowicz and Ziemlański 1992, 1999]. Recently, the attention has been paid to antioxidative properties of other compounds belonging to carotenoids, particularly to lycopene and lutein. It has been shown that lycopene is the most effective compound removing singlet oxygen, and its high consumption is negatively correlated with some types of cancer [Wąsowicz and Gromadzińska 2005, Omoni and Aluko 2005]. First of all, lutein plays some role in protection of retina against harmful action of free radicals and in prevention of atherosclerosis [Boileau 2002, Boban 2002].

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Bioactivity of selenium was proved, in practice, in the second part of last century. It is known as connected with the presence of this element in glutation peroxidase ($GSHP_x$), which plays main protective role against oxidation of cell membranes lipids and takes part in hydrogen peroxide (H_2O_2) and lipids' hydroxyperoxides metabolism. In these processes, selenium plays the role similar to vitamin E, and sometimes can substitute vitamin in its function. Acting as antioxidant, it protects cell membranes against free radicals generation, and thanks to that, the risk of cancer and cardiovascular diseases decreases [Wąsowicz and Gromadzińska 2005].

For several years, big interest of scientists and food producers has been concentrated on phenolic compounds, belonging to natural non-nutritional substances (NSN). In relation to the structure of basic carbon frame, they can be very generally divided to phenolic acids (derivatives of hydroxybenzoic acid and hydroxycinnamic acid) and flavonoids. Among these latter, there are many subclasses, in dependence on the structure of heterocyclic carbon ring [Bravo 1998, Nijveldt 2001, Manach et al. 2004]. Among particular subclasses there is a huge diversity in relation to the number, and location of hydroxyl (OH) groups, forming of metoxyl (OCH_3) groups and substitution of glycoside residues. Detailed chemical structure of many phenolic compounds is not yet well known. However, it is known that chemical and physical properties, biological activity and metabolism depend on the number, kind and location of substituents in molecule. Location and level of hydroxylation have essential influence on antioxidative properties, however the presence in ring B of hydroxyl groups in *orto*- position increases this activity [Scalbert and Williamson 2000, Manach et al. 2005].

Polyphenols are considered to be the most effective antioxidants, they can also intensify the activity of other antioxidants, *inter alia* soluble in lipids vitamins, and also vitamin C. The most popular polyphenols are flavonoids, among which quercetin, kaempferol and apigenin glycosides dominate.

First of all, natural antioxidants are present in plants, and this is why the basis source of these compounds for humans are plant-derived products. Vitamins soluble in lipids and selenium occur also in food derived from animals (milk and fish lipids, eggs), but in smaller amounts, and in dependence on kind of feed consumed (f.e. carotenoids content in milk lipids, eggs). That is why, products derived from animals are not significant sources of antioxidants in human diet. The richest sources of antioxidants are fruits, vegetables, and their preserves, cereals and legumes, tea, coffee, wine, beer, and also herbs and spices.

ANTIOXIDATIVE PROPERTIES OF FRUITS

Fruits belong to food products appreciated by dieteticians and nutritionists. They contain several vitamins and mineral salts deciding about their nutritional value, and also dietary fiber. Majority of fruits are rich source of vitamin C, carotenoids and polyphenolic compounds. Especially berry fruits are precious in this regard. Among berry fruits, the content of particular substances with antioxidant activity is rather diversified – some of them are especially rich source of vitamin C, the other contain huge amounts of phenolic compounds. Fruits of blackcurrant have a high content of antioxidants. They contain huge amounts of vitamin C, in range of 120-215 mg/100 g [Hägg et al. 1995, Benvenuti et al. 2004], and also significant amounts of carotenoids, mainly lutein and

β-carotene [Olsson et al. 2004]. In blackcurrant fruits phenolic compounds are also present, on the level of about 25 mg/g d.m, with anthocyanins consisting 1/3 [Kähkönen et al. 2001, Benvenuti et al. 2004]. Except anthocyanins, blackcurrant fruits contain phenolic acids, among which derivatives of hydroxycinnamic acid dominate, especially m-coumaric acid [Zadernowski et al. 2005].

The subject of many studies were antioxidative properties of strawberry fruits, which are rich source of vitamin C (35-104 mg/100 g), and phenolic compounds [Hägg et al. 1995, Hakala et al. 2003, Klopotek et al. 2005]. Phenolic compounds are present in these fruits in amount about 20 mg/g d.m., where the biggest share have anthocyanins and ellagic acid with its derivatives (glycosides, ellagitannins), which can make 35-40% of total polyphenols content [Kähkönen et al. 2001, Klopotek et al. 2005]. Among anthocyanins, present mainly in pulp, pelargonidin 3-glucoside dominates, while in stones – cyanidin 3-glucoside [Aaby et al. 2005]. Ellagic acid makes up also over a half of polyphenols amount in raspberries [Anttonen and Karjalainen 2005].

Aronia malanocarpa fruits are considered as the richest polyphenols source. Their total content is 40-70 mg/g d.m., with over 50% share for anthocyanins. The latter, contain group of polyphenols are the derivatives of hydroxycinnamic acid, represented mainly by chlorogenic and neochlorogenic acids. These compounds, together with epicatechins, decide about very tart taste of *Aronia malanocarpa* fruits, which causes that they are rarely consumed in raw state [Niedworak and Brzozowski 2001, Wu et al. 2004, Olsson et al. 2004, Oszmiański and Wojdylo 2005]. Phenolic compounds present in grapes, mainly in seeds and peel, are mainly anthocyanins and derivatives of hydroxycinnamic acid, flavonols and stilbenoids. The richest source are pomace received during vine production. Among polyphenols present in seeds of grapes dominate: gallic acid, catechins and epicatechins, while in peel – ellagic acid, myricetin, quercetin, kaempferol i trans-resveratrol [Pastrana-Bonilla et al. 2003, Kammerer et al. 2004]. It was shown, that this last compound is a very active antioxidant, which modulates lipid metabolism, inhibits lipoproteins oxidation and platelets aggregation, what is very important in atherosclerosis prevention. Its presence in red wine is an explanation of French paradox. Resveratrol influences the inhibition of tumour growth and metastasis of malignant tumours. From grape kernels para-pharmaceutic preparations recommended in prevention of ischaemic heart diseases are produced [Lutomski and Mścisz 2003].

The results of many studies showed that the best source of compounds with antioxidant activity are wild plants fruit. Bilberries, being very popular fruits in our country, contain vitamin C and carotenoids, but the most important antioxidants are phenolic compounds. Total content of phenolic compounds in bilberries reaches about 30 mg/g d.m., from this 70% consist anthocyans, and next in order (10%) – derivatives of hydroxycinnamic acid [Kähkönen et al. 1999, 2001, Vinson et al. 2001]. Minor content of antioxidant compounds contain "high" blueberry known as "American", which is contemporary one of more preferably cultivated in Poland and so its significance as a source of antioxidants in our diet increases [Halvorsen et al. 2002, Taruscio et al. 2004].

The rich source of phenolic compounds (about 20 mg/g d.m.) are cranberries. They contain anthocyanins (peonidin and cyanidin), flavanones and procyanidin, and from flavonols – quercetin, myricetin and derivatives of hydroxycinnamic acid [Kähkönen et al. 1999, 2001, Taruscio et al. 2004, Määttä-Riihinen et al. 2004]. It is shown that biologically active phenolic compounds present in these fruit thanks to antioxidative properties decrease risk of atherosclerosis and inhibit tumorous cell growth. Fresh juice from

cranberry fruits (which can the gained from frozen fruits) showes particularly high activity [Rodowski 2001, Ferguson et al. 2004].

Crowberry fruits are valuable source of vitamin C, with its amount about 18 mg/100 g. They contain also carotenoides – lutein (about 3.6 μg/g d.m.) and β-carotene (2 μg/g d.m.) and also phenolic compounds (26-46 mg/g d.m.) [Kähkönen et al. 1999, 2001, Halvorsen et al. 2002, Olsson et al. 2004]. Among them, flavanols and procyanidins, cinnamic acid, trans-resveratrol and p-coumaric acid dominate. They decide about organoleptic features and healing properties of products made from these fruits [Kähkönen et al. 2001, Määttä-Riihinen et al. 2004, Ehala et al. 2005].

Polyphenolic compounds (about 23 mg/g d.m.) of blackberry first of all decide about its antioxidant activity [Benvenuti et al. 2004, Reyes-Carmona et al. 2005]. In their composition, besides anthocyanins and flavonols placed mainly in pulp, ellagic acid has the biggest share and the next in turn are procyanidins and epicatechins stored in seeds [Benvenuti et al. 2004, Siriwoharn et al. 2004, Siriwoharn and Wrolstad 2004, Bushman et al. 2004, Reyes-Carmona et al. 2005, Zadernowski et al. 2005].

Citrus fruits – grapefruits, lemons, oranges – are a rich source of antioxidants, because of big content of vitamin C (40-50 mg/100 g) and phenolic compounds, among which flavanones (hesperitin, naringenin, eriodictyol) dominate. Pink grapefruits show relatively high content of lycopene, too [Gorinstein et al. 2001, Lugasi et al. 2003, Cieślik at al. 2006, Saura-Calixto et al. 2007].

Many authors emphasise the significance of apples as a source of phenolic compounds (even to 5.0 g/kg), with their content about 7-times higher in peel than in pulp. Almost 80% of apple polyphenols contain polymeric procyanidins and monomeric flavanols, with dominating epicatechin and its dimmer procyanidin B2. Next, according to the amount, are phenolic acids, dihydrochalcons and flavonols. Main phenolic acid in apples is chlorogenic acid, among dihydrochalcons – phloridzin and phloretin-2-xyloglucoside. These compounds influence juice quality (especially colour and taste) in a wide range [Lu and Foo 1997, 2000].

VEGETABLES AS A SOURCE OF ANTIOXIDANTS

Among vegetables the best sources of antioxidants are tomatoes, red pepper, *Brassica* vegetables, onion, garlic and red beet. Red pepper is appreciated mainly because of high content of vitamin C (144 mg/100 g) and cryptoxantin, and tomatoes as a source of lycopene. Lycopene is present in tomatoes peel in amount of 3025 μg/100 g, however, considerably higher amounts, and better absorbed form is supplied by tomatoes preserves, for example ketchup (9900 μg/100 g) [Horbowicz and Saniewski 2000, Lugasi et al. 2003].

Considering vitamin C or β-carotene content, parsley roots, kale, carrot and pumpkin are listed among vegetables [Holden et al. 1999, Kopsell and Kopsell 2006]. Tomatoes are also a source of polyphenol compounds, mainly flavonols. Steward at al. [2000] studies show that 98% of flavonols present in tomatoes are placed in fruit peel, from which 96% of it consists the quercetin. In pulp and seeds compounds of quercetin consist about 70%, while kaempherol – 30% of total flavonols content. So it means, that removing of tomato peel significantly decreases polyphenols level.

Brassica vegetables such as white cabbage, kale, Chinese cabbage, broccoli sprouts, kale or cauliflower are known for their pro-healthy properties which are *inter alia* caused by presence of antioxidant compounds in high amounts. They contain vitamin C, in the amount – in dependence on gender – to a few dozen mg/100 g. Kale contains a high amount of this ingredient. It is considered that a higher content of vitamin C among vegetables is met only in red pepper. Kale is a good source of carotenoides (17-34 mg/100 g), and – in opinion of some scientists – surpasses such vegetables as carrot, tomatoes or spinach [Kurilich et al. 1999, Kopsell et al. 2004]. Plants of cabbage vegetables family contain also phenolic compounds, where particularly rich sources are: kale, broccoli and Brussels sprouts. Other vegetables from this group contain smaller (even several times) amounts of these compounds, but thanks to high consumption they are their significant source in everyday diet. *Brassica* vegetables contain also derivatives of hydroxycynnamic acid – caffeic, chlorogenic, ferulic, and synapic [Vallejo 2003] as well as flavonols.

Generally, the flawonoids group dominate among vegetable polyphenols. Bahorun et al. [2004] found that flavonoids share in total content of polyphenols in 6 on 10 studied vegetables was in range of 51-79%. It is worth to be marked, that in non-processed vegetables flavonoids are rarely present in aglycon form. Free quercetin was determined in tomatoes and different kinds of onion. Free kaempherol was present only in tomatoes and free isoramnetin in onion [Price et al. 1997, Stewart et al. 2000, Marotti and Piccaglia 2002]. Among glycosides of flavonol in onion were identified: 4-glucoside of quercetin and 3.4-glucoside of quercetin consisting from 83 to 93% of total polyphenol amount [Price et al. 1997, Marotti and Piccaglia 2002]. Derivatives of quercetin were found also in lettuce [Romani et al. 2002]. Main polyphenol compounds of broccoli are quercetin 3-sophoroside of and kaempferol 3-soforoside [Price et al. 1998].

Over 20 compounds of quercetin and kaempferol were found in cabbage [Nielsen et al. 1998]. In red pepper, two derivatives of quercetin, tree – luteolin and one – apigenin were found [Materska and Perucka 2005]. Anthocyanin pigments are found only in a few vegetables. They give characteristic colour of red cabbage, onion and lettuce with red leaves. They can also be found in peel of radish, aubergine and coloured potatoes. As regarding potatoes, varieties with pink or violet coloured pulp are also cultivated.

Anthocyanin pigments found in vegetables are acyl derivatives of cyanidin (red cabbage, red onion, radish and lettuce), pelargonidin (radish and potatoes) and delfinidin (aubergine) [Clifford 2000]. From phenolic acids group in vegetables are mainly spread derivatives of hydroxycynamonnic acid. Chlorogenic acids in potatoes constitute 90% of all phenolic compounds. In the case of carrot, the amount of chlorogenic acid depends on vegetable colour – with the highest amount in carrot with purple colour, and the lower – in yellow and white. Chlorogenic acid is present also in aubergine and tomatoes. Neochlorogenic acid is present in high amount in broccoli. In carrot, besides chlorogenic acid, caffeic acid and its derivatives were identified [Alasalvar et al. 2001, Mattila and Hellström 2007].

POLYPHENOLS OF CEREALS AND LEGUMES

The cereal grains and legumes are not very rich sources of antioxidant compounds – in practice only phenolic compounds can be found there, but they are mentioned be-

cause of these sources huge share in diet. Among polyphenols enclosed in cereal grains, phenolic acids play important role, and especially ferulic acid is dominating in grains (in wheat and rye, first of all). Besides this compound, vanillic and p-coumaric acids play important role, even though they are present in smaller amounts. In the case of oats, the presence of other polyphenols called avertramidin were recorded, while rutin is the main polyphenol of buckwheat. Phenolic acids are present in two forms: ester and glycoside ones. The chemical structure as well as concentration of these compounds influence their antioxidant activity. The cereal grains are also a source of catechins; the higher amounts of these compounds were found in seeds of buckwheat, next – in oats and rye, and at least in wheat [Holasova et al. 2002, Peterson et al. 2001].

Catechins are present also in seeds of beans, which contain (in dependence on variety) phenolic acids (ferulic, sinapic), quercetin, tannins and anthocyanins (coloured bean), and also isoflavons (genistein, daidzein, glycitein), of which soybean is especially a rich source [Drużyńska and Klepacka 2004, Amarowicz and Troszyńska 2005].

ANTIOXIDATIVE PROPERTIES OF ANIMAL-DERIVED FOOD

Important group of natural antioxidants in animal-derived food products are aminocompounds: aminoacids, peptides and proteins. Antioxidant activity of these compounds is connected mainly with aminoacids which possess thiol groups (metionine, cysteine). Proteins, acting as antioxidants, scavenging of free radicals formed in biochemical processes of cells [Yamamoto et al. 1998, Decker et al. 2000]. Antioxidant activity of proteins from animal-derived products can be also connected with addition (in food technology) of concentrates and isolates gained from high-protein plants origin (legumes seeds) and animal origin (milk, eggs) raw materials. Isolated soybean proteins, because of their good functional properties, are wide used in meat industry and they can inhibit reaction of lipid oxidation [Ulu 2004]. Huge antioxidant activity have also preparations of albumin and globulin derived from seeds of legumes cultivated in Poland – bean, peas, and broad bean. In several studies, the ability of casein and whey proteins of milk to inhibit autooxidation of lipids was confirmed. Casein inhibits enzymatic, as well as non-enzymatic oxidation of lipids [Yamamoto et al. 1998].

Main non-protein thiol compound of animal tissues is glutathione (GSH). Its basic function in organism is protection of thiol protein groups from oxidation. Reduced form of glutathione is a scavenger of RFT. It is worth to add, that glutathione and other reduced thiol compounds can regenerate oxidized α-tocopherol radical to vitamin E [Decker et al. 2000].

In lipid tissue of animal organisms, the compounds that show the activity of vitamin E (tocopherols and tocotrienols), carotenes and ksantophils and ubiquinone (coenzyme Q) are present. Tocopherols, among which α-tocopherol is dominant compound, are located mainly in 2-layer phospholipid structure of cellular membranes, and decide (with cholesterol) about their integrity. They posses ability to scavenge of radicals such as hydroxyl, alcoxyl, peroxyl as well as hydroxyperoxide anion radical, and ability to quench of singlet oxygen. Animals and humans can not synthetise carotenoids, but have only the ability to metabolise them; classic example is reaction of carotene conversion to retinol (vitamin A). In animal organism, carotenoids are located mainly in the liver, they are also present in lipid tissue and blood lipoproteides (LDL and HDL) [Surai 2003].

BEVERAGES AND SPICES AS ANTIOXIDANTS' SOURCES

The group of products which can supply high amounts of antioxidants are beverages, such as: coffee, tea, cocoa, red wine, beer as well as herbs and spices.

Main phenolic compounds present in tea are catechins. Their content is quite diversified in dependence on gender, and technology of raw material preservation. Generally, green tea contains more of these compounds than black or red tea and thanks to this it shows over 2-times higher antioxidant activity [Cao et al. 1996, Droesti 2000, Fik and Zawiślak 2004, Waszkiewicz-Robak et al. 2005].

The content of phenolic compounds in roasted coffee reaches 8%, from which chlorogenic acid is dominant. Infusion from 5 g of milled roasted coffee can contain even about 140 mg of this compound, which can the responsible for possible acrid effect of this drink [Budryn and Nebesny 2005]. Phenolic compounds are present in huge amounts also in cocoa seeds, they consists 12-18% d.m. of seeds. Procyanidins consist about 60% of them. In significantly lover amount, also quercetin and its glycosides are present. Above mentioned flavonols and procyanidins are present also in chocolate, with their content depending on percentage share of cocoa, that is on the kind of chocolate (dark chocolate – 170, white chocolate – 70 mg of flavonoids/100 g) [Bonvehi and Coll 1997, Gajewska and Myszkowska-Ryciak 2006].

Antioxidants present in beer are mainly phenolic compounds which source is barley malt as well as hop. It is calculated that 80% of beer phenols is derived from malt, and about 20% – from hop. To the most important phenolic compounds present in beer belong: phenolic acids (cinnamic, chlorogenic, vanillic, ferulic, gallic, caffeic, o- and p--coumaric, syringic), derivatives of flavan-3-ol (catechin, epicatechin, procyanidin prodelphinidin), flavonoglycosides. The chemically most reactive phenolic compounds of beer are derivatives of flavan-3-ol [Walters et al. 1997, Sieliwanowicz 1998]. A very good source of antioxidants is red wine, that contains many valuable fruit peel-derived polyphenols (resveratrol), which bioavailability increase as the effect of technological process [Droesti 2000, Howard et al. 2002, Rupasinghe and Clegg 2007].

Among the sources of phenolic compounds in food, herbs and spices cannot be omitted. For a long time many plant spices have been a valuable additive to food, playing not only taste forming role and giving smell features, but also in assuring longer shelf-life of food. In this range, the activity of many seasonings, mainly spices is the effect of phenolic compounds content. Strong antioxidative properties show such spices as clove, cinnamon, ginger. In our country there is a long tradition of marjoram and cumin using to fatty and stodgy dishes. Average spices consumption in Europe is about 0.5 g/24 hr, but in Asia and North Africa it is many times higher. For example, in Hindu cuisine, about 60 of different spices are used. In recent years in Poland a higher consumption of these products has been observed. It can be connected *inter alia* with the increase of popularity of various world corners cuisines: Oriental, Mexican, North African or Mediterranean and wider availability of spices on our market. It was became fashionable to cultivate some spice plants in our gardens or pots at home [Hozyasz and Chełchowska, 2005, Hinneburg et al. 2006, Suhaj 2006].

ANTIOXIDANTS IN DIFFERENT NATIONS DIETS

The levels and kinds of antioxidants present in diet depend mostly on the kind of products and the amount in which they are most often consumed. The studies conducted in the USA showed that the main source of antioxidants in the American diet are fruits and vegetables, where 26% of poliphenols is supplied and 25% of total antioxidants level derives from oranges. Generally, the consumption of polyphenols is at the level of about 1 g/day, from which 45% are biflavons, in 20% – catechins and in 17% – anthocyanins [Chun et al. 2005]. In the Finnish diet, a high level of anthocyanins is observed, reaching up to 200 mg/day as the result of a very big consumption of berry fruits rich in these compounds: cranberries, bilberries. In the Netherlands, significant consumption of flavonol monomers (50 mg/day) is observed, mostly from tea, chocolate, apples and pears. Notably a high consumption of catechins and proanthocyanidins supplied with apples, pears, grapes and red wine is recorded in Spain. In France, daily polyphenols intake is at the level of 1 g. About 28% of this value is supplied by fruits and vegetables (mainly apples and patatas) and the rest – from coffee, tea, wine, beverages and cereals. Drinking coffee has a very important effect on the consumption of polyphenols from the group of hydroxycinnamic acids in all countries. Consumption of a few cups daily can supply up to 1000 mg of chlorogenic acid. The diet of Asian countries is rich in isoflavonoids (20-45 mg/day), which is connected with a high consumption of soybean (10--35 g/day) [Kim and Kwon 2001, Beecher 2003, Manach et al. 2004, Brat et al. 2006].

The consumption of phenolic compounds in Poland is not well known yet. There are many products on the market which can supply them. The potential sources in our diet can be potatoes, *Brassica* vegetables, coffee, tea, apples, beverages and beer.

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ŹRÓDŁA NATURALNYCH PRZECIWUTLENIACZY

Streszczenie. Intensywne procesy oksydacyjne zachodzące w organizmie człowieka prowadzą do powstawania reaktywnych form tlenu, które mogą uszkadzać komórki i tkanki ustrojowe. Stwierdzono, że endogenny system ochronny organizmu może być w tym wypadku wspierany przez naturalne związki antyoksydacyjne dostarczane z żywnością. Dokonano oceny produktów spożywczych jako potencjalnych źródeł antyoksydantów, z uwzględnieniem rodzaju dostarczanych przez nich związków i ich znaczenia w diecie różnych nacji.

Słowa kluczowe: antyoksydanty, polifenole, źródła antyoksydantów w diecie

Accepted for print – Zaakceptowano do druku: 16.01.2008

For citation – Do cytowania: Sikora E., Cieślik E., Topolska K., 2008. The sources of natural antioxidants. Acta Sci. Pol., Technol. Aliment. 7(1), 5-17.