Low Molecular Weight Antioxidants and Other Biologically Active Components of Buckwheat Seeds

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ABSTRACT

The biological bases for intake of low molecular weight antioxidants and other biologically active compounds present in the buckwheat seeds in relation to the reduction of risk for chronic diseases have been discussed in this article.

Keywords: buckwheat, chronic diseases, d-chiro-inositol, fagopyritols, flavonoids, glutathione, melatonin, phenolic acids, phytic acid, phytosterols, oxidative stress, vitamins

Abbreviations: GSH, reduced glutathione; IP6, inositol hexaphosphate; LMWA, low molecular weight antioxidants; T, tocopherols (α-T, β-T, γ-T, δ-T); TEAC, Trolox equivalent antioxidant capacity; TPC, total phenolic compounds

INTRODUCTION

Nutritional issues highlight the relationship between diet and chronic diseases such as obesity, heart disease, and cancer (Anderson et al. 2000). Looking at this problem, we find a number of dietary factors that increase health risks, such as elevated intake of energy, especially fat, high consumption of roasted, smoked or salted products, and others. Nowadays, healthiness is considered one of the key drivers in food business. There is an increasing demand for foods having some healthy characteristics (Fogliano and Vitagliano 2005). Many people believe that oxidative stress plays a major role in causing diseases. Oxidative stress occurs when the production of reactive oxygen species goes beyond the antioxidant capability of the target cell.

Humans are subjected to many forms of oxidative stress, including pollutants, radiation, ingestion of oxidized foods, ischemia and in vivo production of free radicals (Halliwell et al. 1995). If unchecked, oxidation can promote a chain of chemical reactions producing free radicals, peroxides and secondary oxidation breakdown products which in turn react with and cause damage to cellular membranes, proteins and nucleic acids. Biological systems control these oxidative factors by a variety of antioxidative mechanisms that restrict the reactivity of reactive oxygen and nitrogen species and oxidation catalyst. The accumulation of oxidative damage has been implicated in both acute and chronic cell damage. In addition, oxidative damage to tissues is modulated by many factors including substrate composition, oxygen, prooxidants and antioxidant concentrations.

Antioxidant compounds may contribute to the health potential of food (Rice-Evans et al. 1997). Antioxidants are compounds that delay the onset or slow down the rate of oxidation of oxidizable substrates and therefore they play an important role in the defence system of the body against reactive oxygen species. Antioxidants can act directly as reducing agents donating protonic hydrogen to the unpaired oxygen electron or by stabilizing or relocating the free radical electron (Halliwell et al. 1995). Some antioxidants trap or scavenge the free radicals, and in the process become prooxidants and antioxidant concentrations.

Nature of antioxidants

Antioxidants, such as ascorbic acid (vitamin C), vitamin E, β-carotene and other carotenoids, selenium and wide variety of antioxidant phytochemicals such as simple phenolic com-
pounds, flavonoid glycosides and complex polyphenols formed by the condensation of different phenolic compounds have been recognized as important contributors to the total antioxidant capacity of cells and plasma (Kris-Etherton et al. 2002; Pellegrini et al. 2003; Stanner et al. 2004). Polyphenolic compounds, especially the flavonoids, have recently been shown to be potent antioxidants in cultured cells (Wolf et al. 2008). Flavonoids are also excellent metal ion chelators and prevent copper-catalysed peroxidation of low density lipoproteins (Scalbert et al. 2005). Human studies of flavonoids have also demonstrated effects that can in part be attributed to their antioxidant action (Williamson and Manach 2005). Epidemiological studies indicate that populations with diets high in flavonoids and carotenoids have lower incidence rates of chronic diseases associated with oxidative stress, such as atherosclerosis, diabetes, neurodegenerative diseases, and cancer (Liu 2004). However, there is much disagreement concerning the mechanism of action of polyphenols in vivo. Polyphenols are treated as xenobiotics by the body and rapidly metabolized and conjugated in the intestinal lining and liver. The most flavonoids in the blood are glucoronide, sulphate or O-methylated conjugates and these conjugates are essentially devoid of antioxidant activity (Halliwell et al. 1995). Recent studies confirm that polyphenols have many intracellular effects beyond scavenging free radicals and chelating metal ion (Scalbert et al. 2005). Reactive oxygen and reactive nitrogen species are also potential signaling molecules. Therefore, dietary antioxidants and the importance of synergistic interactions between them, play a key role in a multilevel defence system against radical attack, especially when we move beyond preventing deficiency diseases into promoting optimal health, longevity, and quality (Andlauer and Furst 1998; Serafini 2006). There is growing scientific evidence associating antioxidant-rich diets with a lower incidence of cardiovascular disease, cancers, and age-related degenerative processes. To this purpose, different databases of antioxidant compounds have been published in the last few years which have been shown as a very useful tool to correlate high antioxidant diet with lower incidence of some disease or to a positive modification of disease biomarkers (Serafini et al. 2002; Valtuena et al. 2008). However, focusing research only on single molecules without any information on the efficiency of the network may well have underestimated the impact of antioxidant in oxidative stress prevention (Serafini 2006).

Chemical and physical state of antioxidants

Antioxidant compounds may be naturally present in different forms in the plant microstructure and their localization can be basically classified in the following categories:

1. Low molecular weight compounds, which are free from chemical or physical interaction with other macromolecules.
2. Compounds, which are physically entrapped into different cellular structure.
3. Compounds, which are chemically bound to other macromolecules.
4. Insoluble antioxidant material.

The first group includes antioxidants that can be easily extracted with water or hydroalcoholic mixture: cinnamic and benzoic acid derivatives (Herrmann 1989), flavonoids family (Manach et al. 2004; Ververidis et al. 2007), hydrolysable tannins (Sanchez-Moreno et al. 2003). Tocopherols and ascorbic acid are always present in free form, however the latest after oxidation to dehydroascorbic acid can react with amino group of protein giving protein ascorbate ad- ducts (Hasenkopf et al. 2002).

In the second category are compounds giving non-covalent interaction with food matrix components as well as those physically entrapped in cellular structure: carotenoids as lipoprotein complexes (Faulks and Southon 2001) and flavonoids physically entrapped in pectin network in some foods (Miglio et al. 2008)
Phytochemicals

Phytochemicals are plant substances that may promote good health but are not essential for life (Scalfert et al. 2005). Oxidative stress, which releases free oxygen radicals in the body, has been implicated in a number of disorders including cardiovascular malfunction, cataracts, cancer, and rheumatism. Polyphenolic compound such as flavonoids have received interest due to their antioxidant effect (Rice-Evans et al. 1997). Phenolic compounds are secondary metabolites with different biosynthetic pathway. A large and heterogeneous group of polyphenols including phenylpropanoids, condensed tannins, lignin, and hydroxyxynamic acids, are derived from the same precursor, L-phenylalanine. The second major group represented by hydrolysable tannins has gallic acid as a precursor (Mikulajova et al. 2005). The major portion of phenolic compounds is located in outer parts of grains, where they are involved in defence against ultraviolet radiation, pathogen invasion and in modification of mechanical properties (Fabjan et al. 2003).

Phenolic acids

Phenolic acids as derived from benzoic acid (Fig. 1) and cinnamic acid (Fig. 2, usually occur in conjugated forms and the level of the free form is strictly controlled in living plant tissue. It should be noted that phenolic acids are compounds which can be converted from one kind into another during different physiological processes and in seeds it may vary not only with species or cultivar but even with the seed size. Moreover, phenolics, predominantly ferulic and coumaric acid, play an important role in limiting polysaccharide degradation by exogenous enzymes, where they act as a cross-link between polysaccharides, and between polysaccharides and lignin (Herrmann 1989).

Small quantities of the following free and ester bound phenolic acids were identified in buckwheat groats: vanillic, syringic, ferulic and coumaric (Zielinski et al. 2006). They were found mainly as ester bound phenolic acids and the highest level was noted for syringic and coumaric acid. The content of free and ester bound phenolic acids was twice lower in respect to barley, four times lower in respect to wheat, and from 10 to 20 times lower than in rye and oat (Zielinski et al. 2001). They formed only 0.1% of total phenolic compounds from 4.08 mg g -1 dry matter (DM) (groats) to 0.17, 0.83 and 1.41 mg g -1 DM for extrudates at 120, 160 and 200°C, respectively (Zielinski et al. 2006). It was suggested that the increase in phenolic acid content is primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction, similarly to the lycopene increase after thermal processing of tomatoes (Dewanto et al. 2002). In contrast, other authors did not find any statistically significant changes in total phenolic compound contents after roasting (200°C, 10 min) and extrusion (die temperature of 170°C) of dark buckwheat flour, perhaps due to short processing time at high temperature (Sensoy et al. 2005).

Phenolic compounds have strong antioxidant properties in vitro, associated with their ability to trap the chain-carrying peroxyl radicals with the formation of hydroperoxides, to scavenge free radicals and to chelate ions of transition metals thereby reducing the capacity of metal to generate free radicals in Fenton reaction (Leibovitz and Mueller 1993).

Flavonoids: rutin and quercetin

Rutin (Fig. 3) and quercetin (Fig. 4) levels in buckwheat depend greatly on growth location and cultivar (Oomah and Mazza 1996). Generally, flavonoid content of *F. tartaricum* (about 40 mg g -1 DM) is much higher than that of *F. esculentum* (10 mg g -1 DM) ranked buckwheat (*Fagopyrum* sp.) among allelopathic plants (Oomah and Mazza 1996; Chao et al. 2002; Xuan and Tsuzuki 2004).

Rutin, a flavonoid commonly present in buckwheat leaves, stems, flowers and seedling cotyledons, was detected in low concentrations in groats from dehulled mature achenes of common buckwheat. In common buckwheat groats, rutin level is about 0.20 mg g -1 DM whilst in tartary buckwheat groats is 80.94 mg g -1 DM. Rutin is the most concentrated in bran, particularly if it contains hull fragments (0.7-0.8 mg g -1 DM). In common buckwheat, rutin is mainly located in the hull (0.84-4.41 mg g -1 DM) (Oomah and Mazza 1996). In general, rutin level is much lower in common buckwheat seeds than in other buckwheat species (Shevchuk 1983). Rutin and other flavonoids are UV-B absorbing plant metabolites in order to protect the seeds from the harmful effects of UV-B radiation and dis-
Quercetin, the aglycone of rutin, is present in considerable less amounts than rutin, indicating the hydrolytic activity of enzyme rutinase (Yasuda et al. 1992). Rutinase is highly specific for rutin and is very active during germination. Since the enzyme becomes more active during hydration of buckwheat seed, partly deteriorated grain during a wet season may have higher amounts of quercetin. In common buckwheat groats, level of quercetin is 0.001 mg g⁻¹ DM. Buckwheat hull contained higher level of quercetin (0.009-0.029 mg g⁻¹ DM) (Wijngaard and Arendt 2006). There is evidence that rutin and other flavonoids from buckwheat have many beneficial effects on human health. It was established that rutin antagonizes the increase of capillary fragility associated with hemorrhagic disease, reduces high blood pressure, decreases permeability of the vessels and has an antiedema effects, and reduces the risk of atherosclerosis whereby may reduce coronary heart disease, possibly through the reduction of platelet aggregation and prevention of low-density lipoproteins oxidation (Li et al. 2000; Fabjan et al. 2003). In many cases, flavonoids show a greater efficacy as antioxidants in food systems on a mole-to-mole basis than the antioxidants vitamin C, vitamin E and β-carotene (Rice-Evans et al. 1997).

**Flavanols, proanthocyanidins and tannins**


Buckwheat bran is most concentrated in non-condensed (2.3-3.8 mg g⁻¹ DM) and condensed tannins (5.9-8.6 mg g⁻¹ DM). Condensed catechins, known as proanthocyanidins similarly to phenolic acids, are present in the bran-aleurone layer of buckwheat grains (Przybylski et al. 1998). The bran containing hull fragments contained at least twice the concentration of condensed tannins than bran without hull fragments (Steadman et al. 2001b). The content of proanthocyanidins is higher in flour (1.59 mg g⁻¹ DM) than in the hulls (1.38 mg g⁻¹ DM) (Quettier-Deleu et al. 2000). Soluble oligomeric condensed catechins occur in common buckwheat grain and are responsible for the astringency and affect the colour and biological activity of buckwheat products. Flavanols and proanthocyanidins are lesser known than rutin in buckwheat, but they could also be interesting in the future applications since they are present in the seed. Proanthocyanid oligomers and catechins are an active principles of widely used vasculoprotective drugs such as grape seed extracts (Endotelon®), suggesting possible application of buckwheat bran or flour in this field (Quettier-Deleu et al. 2000).
Flavon C-glicosides

Flavone C-glicosides, a kind of important constituent of the flavonoid family present in foodstuffs and nutraceuticals, have received much attention recently because of their suggested antioxidant and anticancer properties (Zhang et al. 2005; Zhang et al. 2008). They were found in some plants, such as in the tree Pterocapus marsupium, in the fruits of Curcuma and also in common and tartary buckwheat seeds and sprouts (Kim et al. 2007; Watanabe 2007). Orien- tin and homoorientin, a pair of isomeric compounds, and their 4'-deoxy analogues, namely vitexin and isovitexin (Fig. 5) are the main flavone C-glicosides present in buckwheat grain and sprouts (Watanabe 2007; Zielinska et al. 2007b).

Various biological and pharmacological activities have been attributed to these compounds, such as hypotensive, anti-inflammatory, antispasmodic (Prabhakar et al. 1981), antimicrobial (Agnese et al. 2001), antioxidant/free radical scavenging (Bramani et al. 2003; Picerno et al. 2003), radioprotective effects (Hien et al. 2002) and anti-glycation activities (Yamaguchi et al. 2000; Gugliucci and Menini 2002; Lee et al. 2006). Recently, vitexin and isovitexin in the bovine serum albumin (BSA) - glucose system showed strong inhibitory effects on the formation of fluorescent advanced glycation endproducts (AGEs), comparable to that of rutin, one of the most potent natural AGE inhibitors suggesting that their anti-glycation activities may mainly be due to their radical scavenging capacity (Peng et al. 2008).

While the antioxidant activity of rutin and quercetin has been studied in various model systems (Miller and Rice-Evans 1996; Rice-Evans et al. 1996; Re et al. 1999; Pietta 2000; Ahern and O'Brien 2002; Prior et al. 2005; Balasundram et al. 2006), no available information exist in respect to antioxidant activity of buckwheat flavone C-glicosides. The preliminary results indicate for the following hierarchy of antioxidant activity determined against stable ABTS+ radical cations: quercetin > homoorientin > orientin > rutin >>> isovitexin > vitexin and against stable 2,2'-diphényl-1-pircylihydrzyl radicals (DPPH): homoorientin > quercetin > orientin > rutin >>> isovitexin = vitexin (unpublished data). Therefore, there is a need to complete the data of antioxidative activity of flavone C-glicosides, which may be useful in the evaluation of the contribution of these polyphenols to the antioxidative capacity of buckwheat for efficient breeding of highly antioxidative varieties and then for preventing many diseases resulted from the increased intake of buckwheat products.

Flavonoids and flavone C-glicosides and buckwheat processing

When buckwheat is processed, flavonoid levels and therefore antioxidant activity can be affected. The thermal degradation of the main buckwheat groats flavonoids – rutin and isovitexin – was reported when extensive heat treatment was applied during processing (Dietrych-Szostak and Olezek 1999). A reduction of flavonoids concentration of 20% has been reported when buckwheat was heated for 10 min at 150°C, and a reduction of 40% has been determined when buckwheat was heated for more than 1 hr at 150°C. The significant decrease in rutin content was also found after cooking of buckwheat groats (Kreft et al. 2006). The changes in antioxidant capacity of buckwheat and its products followed the changes in flavonoids composition (Zielinska et al. 2007a).

In contrary, flavonoids contents can be increased by producing buckwheat sprouts. Kim et al. (2004) reported that after seven days of seeding, rutin and quercetin content in common buckwheat sprouts were increased to a maximum of 22.36 and 23.12 mg g⁻¹ DM, respectively. The sprouts produced in dark or light showed a high content of homoorientin, orientin, vitexin, rutin and isovitexin. The flavonoids content in sprouts produced under light was almost twice higher than those of sprouts produced in dark (Zielinska et al. 2007b).

Phytic acid

Inositol hexaphosphate (IP6), known as phytic acid, is a naturally occurring polyphosphorylated carbohydrate, which can be found in food sources high in fiber content (Fig. 6).

IP6 concentrated in grains, is a known antioxidant (Graf et al. 1987). In mature buckwheat seeds, phytate is present in vacuole-derived protein bodies located mainly in embryo tissues and in the aleurone layer. Minerals such as Mg, Zn, K, P and Co are mainly stored as phytate in protein bodies (Steadman et al. 2001b). Phosphorous (P) is an integral part of the phytic acid structure in buckwheat, 60-90% of P was in phytic acid (Steadman et al. 2001b). IP6 makes 98% of total inositol phosphates in groats. Apart from IP6, trace amounts of inositol pentaphosphate and inositol tetraphosphate were reported (Zielinski et al. 2006). The phytic acid is concentrated in bran in the milling fraction which is composed of embryo and seed coat. Buckwheat contains 10.0 mg g⁻¹ of phytic acid and enzyme phytase which releases 2.17 μmol of inorganic phosphate min⁻¹ g⁻¹ (Egli et al. 2003). Buckwheat seeds generally contain higher amounts of phytic acid than the grains of legumes and cereals, but less than many other seeds and nuts (Harland and Oberleas 1987). Buckwheat groats are a good source of IP6, approximately about twice richer in it when compared to cereal grains, such as wheat, rye, dehulled oat and barley (Saastamoine et al. 1992; Laszity 1998; Honke et al. 2000). Like wheat bran, buckwheat bran is a concentrated source of phytic acid. The level as 35-38 mg g⁻¹ in bran without hull was reported (Steadman et al. 2001b). While buckwheat bran is also a good source of minerals, there is a concern that the presence of phytic acid could reduce absorption of minerals ingested in the diet, resulted in the label ‘anti-nutrient’. Additionally, at certain pHs, phytic acid interacts with proteins, possibly reducing their bioavailability, and interferes with metalloenzyme activity (Thompson 1993).

The presence of phytic acid as potential ‘anti-nutrient’ may be considered in consumption of large amounts of buckwheat bran for nutritional or medicinal purposes, but beneficial in a balanced diet with adequate proteins and minerals. Moreover, while phytic acid is likely to be a dietary concern for certain vulnerable populations consuming a diet low in protein, a moderate intake as part of a balanced diet may be beneficial.

Thermal processing such as extrusion cooking caused no statistically significant degradation of inositol hexaphosphates, only by 13%, and no relationship was observed between the process temperature and degradation of IP6. The degradation degree of inositol hexaphosphates e.g. during extrusion or baking indicates that this compound is resistant against the hydrothermal treatments (Zielinski et al. 2001).

Phytic acid – due to its chelating properties – binds some macro- and microelements responsible for generation of free radicals (Graf and Eaton 1993), as well as lowers blood glucose, reduces cholesterol and triacylglycerols, and reduces the risk of cancer and heart diseases (Graf and Eaton 1993; Shamsuddin 1995). Therefore, the recent interest in IP6 is due to its antioxidant function as a result of its ability to chelate divalent cations (Sandberg et al. 1989). IP6 inhibits hydroxyl radical (OH⁻) production by chelating iron in the presence of oxygen (O₂) or superoxide anion radicals (O₂⁻) or any reducing agent; it can also maintain the redox potential of iron by accelerating both the reduction of Fe⁺ to ascorbic acid and oxidation of Fe²⁺ by O₂. Therefore, IP6 could reduce the active oxygen species-mediated cell injury via its antioxidant function. Colonic
bacteria produce oxygen radicals in appreciable amounts, and dietary phytic acid may suppress oxidant damage to intestinal epithelium and neighboring cells (Graf and Eaton 1993; Shamsuddin 1995). IP6 has been found to have significant antitumor properties in regard to reduction of tumor growth in both in vivo and in vitro studies for prostate (Agarwal et al. 2003; Singh et al. 2004), breast (Tantivelkul et al. 2003), and colon (Shamsuddin et al. 1988). IP6 has been suggested to be an effective adjunct for neoadjuvant cancer treatment however further in vivo and human studies are needed to evaluate safety and clinical utility of this agent in patients with pancreatic cancer (Somasundar et al. 2004).

Vitamin B and C

Vitamins are a group of organic compounds that are essential in very small amounts for the normal functioning of the human body. Thiamine (vitamin B₁) is known to be strongly adhered to thiamine-binding proteins in buckwheat seeds (Rapala-Kozik et al. 1999) and its bioavailability is uncertain. In common buckwheat the vitamin B₁ is reported as 2.20 μg g⁻¹ DW whilst tartary buckwheat is a twice richer source of this vitamin (Bonafaccia et al. 2003). Riboflavin (vitamin B₂) content in common buckwheat is lower than vitamin B₁ (Bonafaccia et al. 2003). Total B vitamin content, including also pyridoxine (vitamin B₆) is higher in tartary buckwheat groats whose levels ranges from 0.21 to 0.42 mg g⁻¹ DM. Chemically synthesized D-chiro-inositol has lowered elevated plasma glucose in insulin-resistant monkeys, streptozotocin-treated hyperglycaemic rats, and normal rats after administration intravenously or orally (Ortmeyer et al. 1993, 1995). Administering doses of 10 and 20 mg of D-chiro-inositol in the form of natural buckwheat concentrate decreased serum glucose concentrations by 12-19% in streptozotocin-diabetic rats, however, further studies of the effect of D-chiro-inositol on humans are needed (Kawa et al. 2003). Recently it has been reported that D-chiro-inositol enriched tartary buckwheat bran extract lowered the blood glucose level in mice, confirming the beneficial effect of this compound by improving glucose tolerance and insulin response to glucose metabolism without affecting body weight (Yao et al. 2008).

The most D-chiro-inositol in buckwheat is present in the forms of fagopyritols (Horbowicz et al. 1998). Fagopyritols, besides other soluble carbohydrates, are concentrated mainly in the embryo, their concentration is low in endosperm whereas their total content ranges from 1 to 6% (Steadman et al. 2000). Fagopyritol B1 is the most abundant fagopyritol in buckwheat seeds and represents 41.16% of soluble carbohydrates in embryo. In addition to fagopyritol B1, four other fagopyritols have been identified in embryo from common buckwheat seeds: two digalactosyls (fagopyritol B2) and two trigalactosyls (fagopyritol B3) (Steadman et al. 2000). Tartary buckwheat contained 50% of the level of fagopyritols that are present in common buckwheat. Fagopyritols are active substances that may be used in the treatment of diabetes and polycystic ovarian syndrome (PCOS) (Horbowicz et al. 1998; Janet et al. 2005).

Lipophilic LMWA

Buckwheat grains contain from 1.5 to 4% of total lipids (Steadman et al. 2001b), but the content of raw fat in buckwheat flour exceeds 2% (Winggaard and Arendt 2006). The highest concentration of lipids was found in the embryo (7-14%), whereas the lowest in the hull (0.4-0.9%) (Bonafaccia et al. 2003). Holasova et al. (2002) reported that the lipophilic components of buckwheat seeds, e.g. tocopherols and carotenoids do not significantly participate in the antioxidative activity of buckwheat.

D-Chiro-inositol and fagopyritols

D-Chiro-inositol (Fig. 8) is an inositol isomer that occurs in relatively high levels in buckwheat seeds (Horbowicz and Obendorf 2005).

Glutathione

Buckwheat groats are a rich source of glutathione (γ-glutamyl-cysteinyl-glycine; GSH), which is a water-soluble biologically active tripeptide (Fig. 7). Buckwheat groats contained twice higher amounts of GSH (1.10 μmol g⁻¹ DM) than wheat and rye grains, and almost three fold higher amounts when compared to barley and oat grains (Zielinski et al. 2001). This finding is in agreement with recent studies which showed that cereals as well as dairy products were generally low in GSH. From the technological point of view, glutathione has been considered to play an important role in the reduction of hydrogen peroxide and organic peroxides by peroxidases, and it quenches reactive oxygen species. Glutathione is also linked to other antioxidants. It is able to reduce dehydroascorbate to ascorbate, thus recycling the ascorbate. Trolox equivalent antioxidant capacity (TEAC) of reduced glutathione has been reported as 0.90, and it was comparable to 0.97 for α-tocopherol but was lower than derived for phenolic acids as 1.43 for vanillic acid, 1.36 for syringic acid, 1.90 for ferulic acid, 2.22 for coumaric acid, 2.40 for rutin, 4.17 for quercetin aglycone and 1.34 for melatonin (Rice-Evans and Miller 1994; Rice-Evans et al. 1996).

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Vitamin E

In nature, eight substances have been found to have vitamin E activity: α-, β-, γ- and δ-tocopherol (α-T, β-T, γ-T and δ-T); and α-, β-, γ- and δ-tocotrienols (α-T3, β-T3, γ-T3 and δ-T3) (Burton and Ingold 1981). Generally, when the vitamin E activity of a particular food is evaluated, the sum of the eight vitamins is usually quantified taking into account their relative activities together.

Buckwheat does not contain tocotrienols whilst tocopherol (Fig. 9) content was reported as 54.6 μg g⁻¹ DM as maximum amount in buckwheat groats (Zielinski et al. 2001). Zielinski et al. (2001) and Kim et al. (2004) reported γ-tocopherol as the main tocopherol, while Przybylski et al. (1998) detected α-tocopherol. Differences have been attributed to different cultivars of common buckwheat (Przybylski et al. 1998; Zielinski et al. 2001; Kim et al. 2004).

When tocopherols found by Zielinski et al. (2006) in common buckwheat groats were expressed in terms of vitamin E activity and their biological activities were taken into account, buckwheat groats proved to contain smaller amount of vitamin E when compared to wheat, barley and oat, and a comparable level when compared to oat grain (Peterson and Qureshi 1993). Tertiary buckwheat contains higher level of tocopherols than common buckwheat (Kim et al. 2004).

Vitamin E, a major biological antioxidant, quenches free radicals and acts as a terminator of lipid peroxidation, particularly in membranes that contain highly unsaturated fatty acids (Burton and Traber 1990). Another possible mechanism for vitamin E relates to its capacity to keep selenium in the reduced state (Ellis et al. 1984). Vitamin E inhibits the formation of nitrosamines, especially at low pH (Slavin 2000). The bioavailability of dietary vitamin E is affected by differences among ingested forms (Acuff et al. 1994), processing methods, physiological factors (e.g. nutritional status), drugs and other dietary components (Erdman et al. 1988). For example, extrusion cooking caused a significant decrease in vitamin E content by average 63%, mainly due to γ-T thermal degradation (Zielinski et al. 2006). Biological activity of various tocopherols differs and declines in the order: α-T > β-T > γ-T > δ-T, however, their antioxidant activity in vitro has been reported as follow: α-T < β-T < γ-T < δ-T (Belitz and Grosch 1987). Early in vitro studies showed a clear superiority of α-T for trapping peroxyl radicals followed by β-T, γ-T and δ-T. Their relative reactivities with peroxyl radicals were 100/60/25/27 (Burton and Ingold 1981) and seemed to account in large part for their relative vitamin E activity. However, later studies showed that their relative reactivities in trapping singlet oxygen were 100/100/76/34 for α-T/β-T/γ-T/δ-T (Kaiser et al. 1990), while γ-T was reported to be superior to α-T for quenching nitrogen dioxide and peroxy nitric radicals (Cooney et al. 1993; Christen et al. 1997). The provided evidences show that antioxidant activity of various tocopherols in vitro seems to depend on the kind of reactive oxygen species and sources generating such species.

Carotenoids

Carotenoids are not significant antioxidants originated from buckwheat seeds since very low level of 2.10 μg g⁻¹ DM was reported by Gabrovská et al. (2002).

Phytosterols

The distribution and content of phytosterols in the whole buckwheat vary significantly from different tissues and differing growing phases (Li and Zhang 2001). The most abundant phytosterol is β-sitosterol followed by campesterol and trace of stigmasterol (Fig. 10). According to Horbovicz and Obendorf (1992), the content of sterols in de-hulled groats after lipid extraction was 0.70 mg g⁻¹ DM for β-sitosterol and about 0.09 mg g⁻¹ DM for campesterol. The similar distribution was reported in buckwheat flour which contained 0.86 mg g⁻¹ DM of β-sitosterol, 0.11 mg g⁻¹ DM of campesterol and 0.02 mg g⁻¹ DM of stigmasterol (Normen et al. 2002). The embryo and endosperm tissues are the most rich in phytosterols. No presence of sitostanol and campestanol in buckwheat was reported.

The phytosterols found in buckwheat, although at a low level, show positive effects in lowering blood cholesterol level. The ingestion of phytosterols significantly inhibits the absorption of cholesterol in vivo. Phytosterols, particularly β-sitosterol, which cannot be absorbed in human body, have a similar structure with cholesterol and can show strong competitive inhibitory effect on cholesterol absorption in vivo (Krkošková and Mrázová 2005). Phytosterols as antioxidants in food were reported as inhibitors of edible fat oxidation during storage and frying (Gordon and Magos 1983; Boskou and Morton 1976).

Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) (Fig. 11), the principal hormone of the pineal gland of vertebrates, is implicated in several physiological processes controlled by photoperiod like diurnal rhythm of locomotor activity as well as seasonal rhythm of reproduction.

It has been demonstrated that edible plant tissues contain melatonin and their consumption increases the melatonin level in the circulating blood in vertebrates (Hattori et al. 1995). Moreover, the presence of radioimmunoassayable melatonin in feverfew (Tanacetum parthenium (L.) Sch. Bip.) and other medicinal plants has been detected (Murch et al. 1997).

The melatonin level found in common buckwheat groats (470 pg g⁻¹ DM) was comparable to those of cereal grains (200-700 pg g⁻¹ DM) (Zielinski et al. 2001, 2006). The amount of melatonin after thermal treatment – extrusion cooking of buckwheat groats was decreased up to by 63% (Zielinski et al. 2006). The amount of melatonin found in groats and extrudates was lower than that found previously in medicinal plants (Murch et al. 1997), however, it was higher than that found in edible-plant products (Hattori et al. 1995). Moreover, considering the potent antioxidant activity of melatonin and its synergistic positive interactions with...
Antioxidants such as Trolox, glutathione, ascorbate in aqueous system (Poeggeler et al. 1995) and with α-T in a lipid bilayer (Livrea et al. 1997), may be concluded that ingestion of buckwheat-based products selected for their melatonin content may play a role in the protection against radi cal-mediated cellular damage as well as cancer progression and promotion in vivo. Melatonin possesses remarkable antioxidant properties in vivo, and its activity in vitro suggests that it acts primarily as a trap for free radicals with antioxidant properties. Therefore, melatonin is known to be rapidly taken up from aqueous solutions such as Trolox, glutathione, ascorbate in aqueous media. Melatonin has been shown to be rapidly taken up from the gastrointestinal tract when it is administered orally. A dose of synthetic melatonin of 1.0 to 1.5 μg kg⁻¹ is sufficient to raise the daytime blood melatonin levels to high values normally seen in humans at night (Vakkuri et al. 1985). In view of its ability to cross all morphological barriers, melatonin is potentially an important component of the anti-oxidative defence system of the organism.

SUMMARY
Antioxidant activity of buckwheat seeds proved to be higher when compared with those of oats, barley, buckwheat straws and hulls. Antioxidant activity of buckwheat is derived mainly from lipophiles whilst lipophobic substances showed only slight antioxidant activity. The highest antioxidant activity was derived from methanolic extract due to the content of phenolics such as 3-flavanols, flavonoids and phenolic acids. It was in agreement with the conclusion of Przybylski et al. (1998), who followed the antioxidant activity of buckwheat grains. Rutin plays an important role in antioxidant activity of buckwheat seeds since correlation between antioxidant activity and rutin content was found in three different buckwheat species: Fagopyrum esculentum, Fagopyrum tataricum and Fagopyrum homotropicum (Jiang et al. 2007). Moreover, buckwheat genotypes from Slovakia (‘Spacinska’ and ‘Pyra’) showed the highest antioxidant capacities by DPPH test and the highest contents of phenolic compounds, being a rich source of flavanoids, mainly rutin, when compared to other cereals and pseudocereals (Mikulajova et al. 2007). Then, the actual value of antioxidants and other biological active buckwheat components can be estimated only after making allowance for losses which occur during technological process (Nicoli et al. 1999), and then during digestion, absorption, and metabolism (Serrano et al. 2007). Since buckwheat seeds contain significant concentration of antioxidative compounds, it is possible that they could contribute to dietary antioxidant intake if these antioxidants are present in active forms in buckwheat-based foods. On the other hand, buckwheat contains biologically active compounds out of the antioxidant activity which must be taken into account for the evaluation of the health benefits of buckwheat based products in human. Such information will increase understanding of the function of buckwheat based products in the diet to reduce chronic diseases.

REFERENCES
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