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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

CONTENTS

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 as one of the key drivers in food business. There is an increasing demand for foods having some healthy characteristics (Fogliano and Vitaglione 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 themselves. For this reason, the role of antioxidants in the reduction risk for chronic diseases is under intensive investigations by many laboratories around the world (Andlauer and Furst 1999; Scalbert *et al*. 2005).

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-

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Invited Review

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 can 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 (Haliwell *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 adducts (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)

In the last two categories, the solubility of the original compounds is completely modified becoming more similar to that of the macromolecule they are linked with. A representative example are phenolic compounds bound to cell wall polysaccharides through ester bonds (McKeehen *et al.* 1999; Bunzel *et al.* 2001; Vitaglione *et al.* 2008), so called "antioxidant dietary fibre" (Saura-Calixto 1998). Insoluble antioxidant material includes at least three large groups of compounds: insoluble proteins, tannins and melanoidins formed after thermal treatment (Hofmann 1998).

Antioxidants from cereals and pseudocereals

Low molecular weight antioxidants in cereals and pseudocereals include nutrients, such as compounds with the vitamin E activity, trace elements being the components of antioxidative enzymes and non-nutrients such as phenolic compounds (phenolic acids, flavonoids, lignans), phytic acid, reduced glutathione and melatonin. The natural antioxidants may have one or more of the following functions: free radical scavengers, reducing agents, potential complexers of prooxidant metals and quenchers of the formation of singlet oxygen. Moreover, many of the natural antioxidants exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombotic, and vasodilatory actions (Cook and Sammon 1996). Many of the biological functions, such as antimutagenicity, anticarcinogenicity, and antiaging, among others, originate from this property (Halliwell *et al.* 1995).

Potential high molecular weight antioxidants in whole grain include polysaccharides and proteins. Cereals contain polysaccharides that inhibit lipoxygenase-catalyzed oxidation of linoleic acid in an emulsions model (Lehtinen and Laakso 1997). While protein and starch might also have an effect on lipid oxidation rates in the phosphatidylcholine model (Baublis *et al.* 2000), their impact on health would likely be less important since they are hydrolyzed during digestion into amino acids and sugars that do not possess strong antioxidant activity. On the other hand, antioxidant compounds can stick to the protein surface by hydrophobic interaction become part of protein-antioxidant complexes (Ramos *et al.* 2008).

FOCUS ON BUCKWHEAT AS A SOURCE OF ANTIOXIDANTS AND BIOACTIVE COMPOUNDS

Buckwheat is an important crop in some areas of the world which refers to any member of *Fagopyrum* family (*Polygonaceae*). There are many species of buckwheat in the world, and mainly nine species have agricultural meaning. Generally, *Fagopyrum* has two groups of species: annual (*Fagopyrum esculentum* Moench, *Fagopyrum tataricum* L. and *Fagopyrum giganteum* Krotov) and perennial species (*Fagopyrum cymosum* Meissn, *Fagopyrum suffruticosum* Fr. Schmidt and *Fagopyrum ciliatum* Jaegt). Among these species, only common buckwheat (*F. esculentum*) is commonly grown, while *F. tartaricum* is grown in some mountainous region.

The fruit of buckwheat is a triangular achene. The hull (pericarp, fruit coat), the outer layer of the achene, has a hard fibrous structure that is usually dark brown or black in colour. Removal of hulls by impact dehulling releases whole groats. The outer part of the dehulled achene is the seed coat, a fibrous layer composed of cells with thickened cell walls and remnants of the nucellus (perisperm). The aleurone, the outermost layer of cells in the endosperm, is a single layer of small cells with thick cell walls. Central endosperm cells have thin cell walls and are packed with starch granules (Steadman *et al.* 2001a). Common buckwheat has softer hull than its *F. tartaricum* relatives. Buckwheat seeds taste harsh for *F. esculentum* and light bitter for *F. tartaricum*.

Buckwheat is presently considered a food component of high nutritional value. Buckwheat has balanced amino acid composition and is rich in lysine (Lys) and arginine (Arg)

(Watanabe 1998). The Lys/Arg and Metionine/Arg ratios in buckwheat proteins are lower than those in the proteins of the most plants. This indicates that buckwheat should be characterised by the properties capable of lowering blood cholesterol level (Sugiyama *et al.* 1985). Both kernel and hull are source of antioxidants and therefore buckwheat appears to be a suitable component of food products from the nutritional aspect and from the point of view of its antioxidant activity, however from the nutrition point of view the buckwheat kernel is the basic source of low molecular weight antioxidants (LMWA). Buckwheat has attracted increasing attention from food scientists for its healing effects over chronic diseases and it has become more popular in many countries as a health food (Li and Zhang 2001; Gabrovska *et al.* 2002). Besides its high-quality proteins, buckwheat is rich in many rare components with healing effects to some chronic diseases. Among them, the most attractive ones are phenolic acids, flavonoids, phytic acid, vitamin B and C, glutathione, D-*chiro*-inositol and fagopyritols, vitamin E, carotenoids, phytosterols, melatonin, and thiamin-binding proteins which serve as B_1 vitamin transporters in the plant and stabilize it during technological processing (Li and Zhang 2001; Wijngaard and Arendt 2006; Christa and Soral-Śmietana 2008).

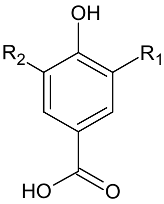
Phytochemicals

Phytochemicals are plant substances that may promote good health but are not essential for life (Scalbert *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, flavonoids and hydroxycinnamic 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.* 2007). 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

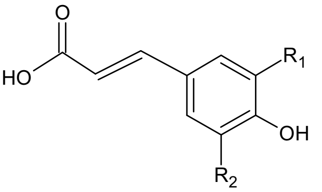
Phenolic acids as derived from benzoic acid (**Fig. 1**) and cinnamic acid (**Fig. 2**), usually occur in conjugated forms and the level of the free form is strictly controlled in living plant tissue. It should be noted that phenolic acids are compounds which can be converted from one kind into another 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 (Zieliński *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 bond 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 (Zieliński *et al.* 2001). They formed only 0.1% of total phenolic compounds in buckwheat, which confirmed that flavonoids and flavonols, including proanthocyanidins, are the main phenolics in buckwheat groats (Watanabe 1998; Dietrych-Szostak and Oleszek 1999; Quettier-Deleu *et al.* 2000).



	R_1	R_2
p-hydroxybenzoic acid	H	H
3,4-dihydroxybenzoic acid	H	OH
vanillic acid	OCH_3	H
syringic acid	OCH_3	OCH_3

Fig. 1 Chemical structures of *p*-hydroxy derivates of benzoic acid.



	R_1	R_2
p- coumaric acid	H	H
caffeic acid	OH	H
ferulic acid	OCH_3	H
synapic acid	OCH_3	OCH_3

Fig. 2 Chemical structures of hydroxy-derivates of cinnamic acid.

The hydrothermal processing of cereal whole grain may release phenolic acids and their derivatives from the cells of fruit wall. Then, the released phenolic acids may contribute higher antioxidant potential when they are considered as a dietary antioxidant. The hydrothermal processing changed the phenolic acid content in the extruded buckwheat groats as an increase in free and ester bound phenolic acids, with the exception for vanillic acid. Following extrusion cooking of the groats at 120, 160 and 200°C phenolic acids made 0.95, 1.31 and 0.84% of total phenolics compounds, respectively, and were masked by the decrease in total phenolics compounds from 4.08 mg g^{-1} dry matter (DM) (groats) to 1.17, 0.83 and 1.41 mg g^{-1} DM for extrudates at 120, 160 and 200°C, respectively (Zieliński *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 metal 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, flavonoids content of *F. tartaricum* (about 40 mg g^{-1} DM) 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, level of rutin 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-

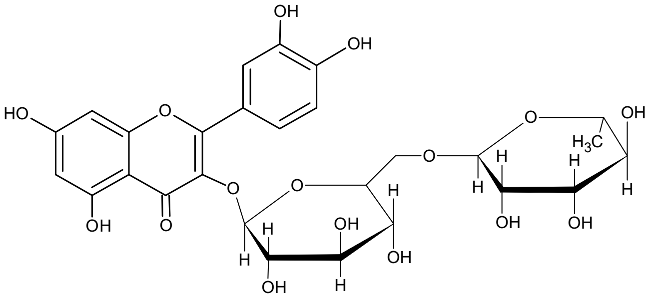


Fig. 3 Chemical structure of rutin (quercetin-3-rutinoside).

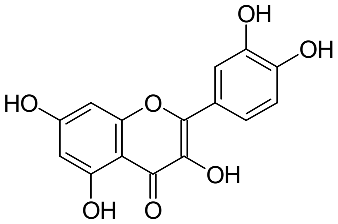


Fig. 4 Chemical structure of quercetin (3,3',4',5,7-pentaxydroxy-flavone).

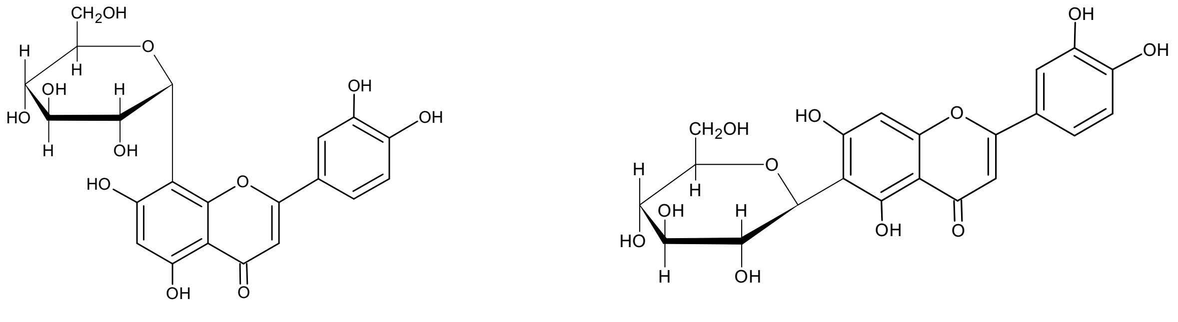
eases (Fabjan *et al.* 2003).

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 hydratation 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 arteriosclerosis 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

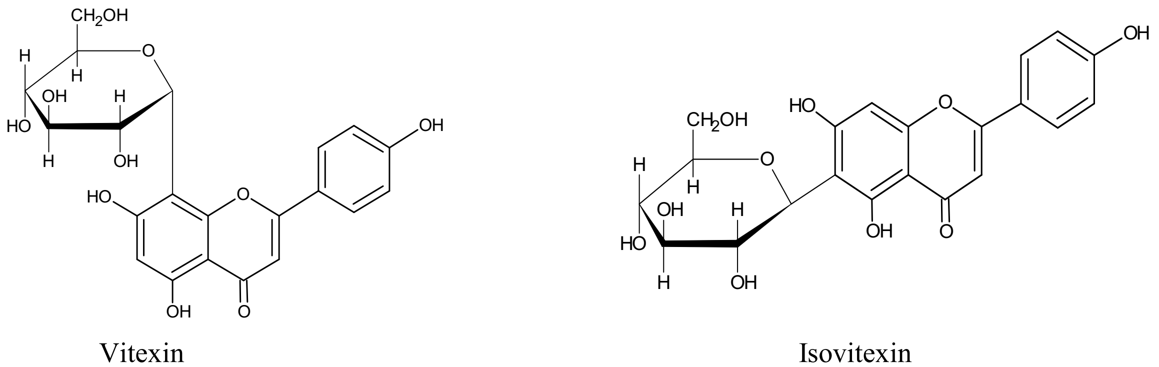
Watanabe (1998) isolated four catechins with antioxidant activity from ethanol extracts from buckwheat groats (*F. esculentum* Moench): epicatechin, catechin 7-*O*-β-D-glucopyranoside, epicatechin 3-*O*-*p*-hydroxybezoate, and epicatechin 3-*O*-(3,4-di-*O*-methyl)-gallate. Catechins show a higher antioxidant activity than rutin (Watanabe 1998).

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. Proanthocyanidin 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).



Orientin
(3',4',5,7-tetrahydroxyflavone-8-glucoside)

Homoorientin
(3',4',5,7-tetrahydroxyflavone-6-glucoside)



Vitexin
(4',5,7-trihydroxyflavone-8-glucoside)

Isovitexin
(4',5,7-trihydroxyflavone-6-glucoside)

Fig. 5 Chemical structures of the flavone *C*-glucosides: orientin, homoorientin, vitexin and isovitexin.

Flavone C-glucosides

Flavone *C*-glucosides, a kind of important constituent of the flavonoid family present in foodstuffs and nutraceutical, 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 Cucurbitaceae, and also in common and tartary buckwheat seeds and sprouts (Kim *et al.* 2007; Watanabe 2007). Orientin and homoorientin, a pair of isomeric compounds, and their 4'-deoxy analoques, namely vitexin and isovitexin (**Fig. 5**) are the main flavone *C*-glucosides present in buckwheat grain and sprouts (Watanabe 2007; Zielińska *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 (Bramami *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; Aherne 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*-glucosides. The preliminary results indicate for the following hierarchy of antioxidant activity determined against stable $ABTS^{\bullet+}$ radical cations: quercetin > homoorientin > orientin > rutin >>> isovitexin > vitexin and against stable 2,2-diphenyl-1-picrylhydrazyl 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*-glucosides, 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-glucosides and buckwheat processing

When buckwheat is processed, flavonoids 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 Oleszek 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 (Zielińska *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 then those of sprouts produced in dark (Zielińska *et al.* 2007b)

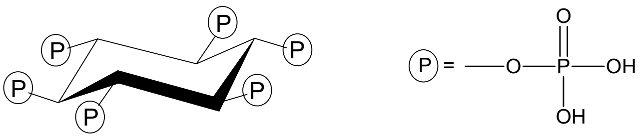


Fig. 6 Chemical structure of inositol hexaphosphate (IP6, phytic acid).

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 (Saastamoinen *et al.* 1992; Lasztity 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 'antinutrient'. 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 'antinutrient' 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 (Zieliński *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 ($OH^{\bullet}$) production by chelating iron in the presence of oxygen (O_2) or superoxide anion radicals ($O_2^{\cdot-}$) or any reducing agent; it can also maintain the redox potential of iron by accelerating both the reduction of Fe^{+3} by ascorbic acid and oxidation of Fe^{+2} by O_2. 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 supress 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 (Tantivejkul *et al.* 2003), and colon (Shamsuddin *et al.* 1988). IP6 has been suggested to become an effective adjunct for pancreatic 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 B_1) is known to be strongly adhered to thiamine-binding proteins in buckwheat seeds (Rapała-Kozik *et al.* 1999) and its bioavailability is uncertain. In common buckwheat the vitamin B_1 is reported as 2.20 $\mu g\ g^{-1}$ DW whilst tartary buckwheat is a twice richer source of this vitamin (Bonafaccia *et al.* 2003). Riboflavin (vitamin B_2) content in common buckwheat is lower than vitamin B_1 (Bonafaccia *et al.* 2003). Total B vitamin content, including also pyridoxine (vitamin B_6) is higher in tartary buckwheat that in common buckwheat. Generally, the quantity of B vitamins is the highest in the bran. Tartary buckwheat bran contains about 6% of daily therapeutic doses of pyridoxine, effective (along with folic acid and vitamin B_{12}) in the reduction of homocysteine levels in blood plasma and in the decrease of the rate restenosis after coronary angioplasty (Krkoškova and Mrazova 2005).

Levels of vitamin C was reported as 50 $\mu g\ g^{-1}$ DM and can be increased up to 250 $\mu g\ g^{-1}$ DM in buckwheat sprouts (Lintschinger *et al.* 1997; Kim *et al.* 2004). Trolox equivalent antioxidant capacity (TEAC) of thiamine (vitamin B_1) has been reported as 0.32, whilst other B vitamins did not show significant antioxidant activity when compared to the antioxidant activity of ascorbic acid (Rice-Evans *et al.* 1996; Gliszczyńska-Świgło 2006).

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 $\mu mol\ g^{-1}$ DM) than wheat and rye grains, and almost three fold higher amounts when compared to barley and oat grains (Zieliński *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 redox reactions in flour and baking technology (Li *et al.* 2005).

The cereals and pseudocereals are generally low in GSH, fruits and vegetables have moderate to high amounts of GSH whilst freshly prepared meats are relatively high in GSH. Frozen foods generally had GSH contents similar to fresh foods, whereas other forms of processing and preservation generally resulted in extensive loss of GSH (Jones *et al.* 1992). The hydrothermal processing of common buckwheat groats such as extrusion cooking caused a significant decrease of GSH, on average by 42% (Zieliński *et al.* 2006).

Glutathione acts as a substrate 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 vanilic 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).

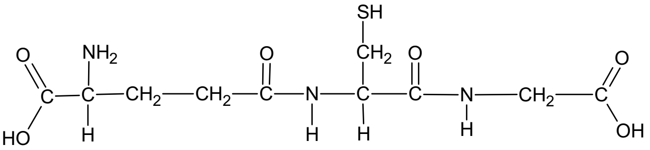


Fig. 7 Chemical structure of glutathione.

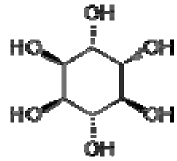


Fig. 8 Chemical structure of D-*chiro*-inositol.

D-*Chiro*-inositol and fagopyritols

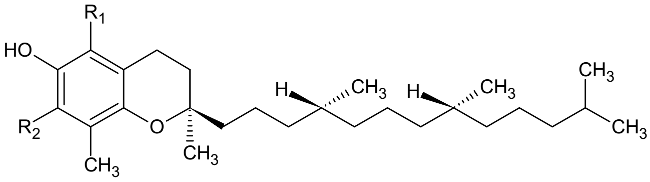
D-*Chiro*-inositol (**Fig. 8**) is an inositol isomer that occurs in relatively high levels in buckwheat seeds (Horbowicz and Obendorf 2005).

Steadman *et al.* (2001a) reported free D-*chiro*-inositol in 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

Buckweat 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% (Wijngaard 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%) (Bonaffacia *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 antioxidant activity of buckwheat.



	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

Fig. 9 Chemical structures of tocopherols.

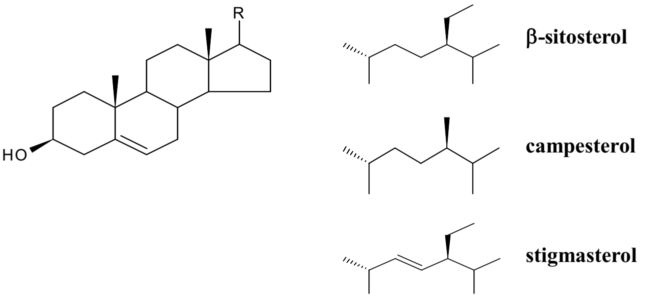


Fig. 10 Chemical structures of sterol (left) and the main buckwheat phytosterols (right).

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 Traber 1990). Generally, when the vitamin E activity of a particular food is evaluated, the sum of the all 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 (Zieliński *et al.* 2001). Zieliński *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; Zieliński *et al.* 2001; Kim *et al.* 2004). When tocopherols found by Zieliński *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). Tartary 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 (Zieliński *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 peroxynitrite 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 Gabrovska *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 sigmasterol (**Fig. 10**). According to Horbowicz and Obendorf (1992), the content of sterols in dehulled 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 can not be absorbed in human body, have a similar structure with cholesterol and can show strong competitive inhibitory effect on cholesterol absorption *in vivo* (Krkoškova and Mrazova 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 parthenuim* (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) (Zieliński *et al.* 2001, 2006). The amount of melatonin after thermal treatment – extrusion cooking of buckwheat groats was decreased up to by 63% (Zieliński *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

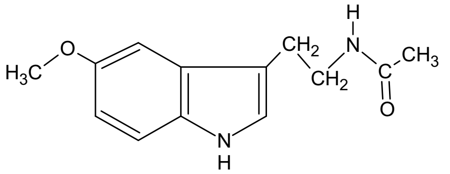


Fig. 11 Chemical structure of melatonin.

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), it may be concluded that ingestion of buckwheat-based products selected for their melatonin content may play a role in the protection against radical 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 an $OH^\bullet$ structure scavenger (Reiter 1995, 1997). Melatonin, which is both hydrophilic and hydrophobic (Shida *et al.* 1994), can enter every cell and all subcellular compartments. Therefore, melatonin is known 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 antioxidative 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 polyphenols whilst lipophilic 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 groats. 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 flavonoids, 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 contain 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

Acuff RV, Thedford SS, Hidiroglou NN, Papas AM, Odeom TA (1994) Relative bioavailability of RRR-and all-rac-α tocopheryl acetate in humans: Studies using deuterated compounds. *American Journal of Clinical Nutrition* **60**, 397-402

Agarwal C, Dhanalakshmi S, Singh RP, Agarwal R (2003) Inositol hexaphosphate inhibits constitutive activation of NFkappa B in androgen-independent human prostate carcinoma DU145 cells. *Anticancer Research* **23**, 3855-3862

Agnese AM, Perez C, Cabrera JL (2001) *Adesmia aegiceras* antimicrobial activity and chemical study. *Phytomedicine* **8**, 389-394

Aherne SA, O'Brien NM (2002) Dietary flavonols: chemistry, food content, and metabolism. *Nutrition* **18**, 75-81

Anderson JW, Hanna TJ, Peng X, Kryscio RJ (2000) Whole grain foods and heart disease risk. *Journal of the American College of Nutrition* **19**, 291S-299S

Andlauer W, Furst P (1998) Antioxidative power of phytochemicals with special reference to cereals. *Cereal Foods World* **5**, 356-360

Andlauer W, Furst P (1999) Does cereal reduce the risk of cancer? *Cereal Foods World* **44**, 76-78

Balasundram N, Sundram K, Samman S (2006) Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence and potential uses. *Food Chemistry* **99**, 191-203

Baublis AJ, Lu C, Clydesdale FM, Decker EA (2000) Potential of wheat-based cereals as a source of dietary antioxidants. *Journal of the American College of Nutrition* **19**, 308S-311S

Belitz HD, Grosch W (1987) *Food Chemistry*, Springer Verlag, Berlin, 310 pp

Bonafaccia G, Marocchini M, Kreft I (2003) Composition and technological properties of the flour and bran from common and tartary buckwheat. *Food Chemistry* **80**, 9-15

Boskou D, Morton I (1976) Effect of plant sterols on the rate of deterioration of heated oils. *Journal of the Science of Food and Agriculture* **27**, 928-932

Bramami L, Aquilano F, Pietta P (2003) Unfermented rooibos tea: quantitative characterization of flavonoids by HPLC-UV and determination of the total antioxidant activity. *Journal of Agricultural and Food Chemistry* **51**, 7472-7474

Bunzel M, Ralph J, Martia JM, Hatfield RD, Steinhart H (2001) Diferulates as structural components in soluble and insoluble cereal dietary fibre. *Journal of the Science of Food and Agriculture* **81**, 653-660

Burton GW, Ingold KU (1981) Autoxidation of biological molecules. I. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants *in vitro*. *Journal of the American Chemical Society* **103**, 6472-6477

Burton GW, Traber MG (1990) Vitamin E: antioxidant activity, biokinetics and bioavailability. *Annual Review of Nutrition* **10**, 357-382

Chao PL, Hsiu S, Hou Y (2002) Flavonoids in herbs: biological fates and potential interactions with xenobiotics. *Journal of Food and Drug Analysis* **10**, 219-228

Christa K, Soral-Śmietana M (2008) Buckwheat grains and buckwheat products – nutritional and prophylactic value of their components – a review. *Czech Journal of Food Science* **26**, 153-162

Christen S, Woodall AA, Shigenada MK (1997) Gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications. *Proceedings of the National Academy of Sciences USA* **94**, 3217-3222

Cook NC, Samman S (1996) Flavonoids – chemistry, metabolism, cardioprotective, and dietary sources. *Journal of Nutritional Biochemistry* **7**, 66-76

Cooney RV, Franke AA, Harwood PJ (1993) Gamma-tocopherol detoxification of nitrogen dioxide: superiority to alpha-tocopherol. *Proceedings of the National Academy of Sciences USA* **90**, 1771-1775

Dewanto V, Wu X, Adom KK, Liu RH (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry* **50**, 3010-3014

Dietrych-Szostak D, Oleszek W (1999) Effect of processing on the flavonoids content in buckwheat (*Fagopyrum esculentum* Moench) grain. *Journal of Agricultural and Food Chemistry* **47**, 4384-4387

Egli I, Davidsson M-A, Juillerat D, Barclay D, Hurrell R (2003) Phytic acid degradation in complementary foods using phytase naturally occurring in whole grain cereals. *Journal of Food Science* **68**, 1855-1859

Ellis N, Lloyd B, Lloyd RS, Clayton BE (1984) Selenium and vitamin E in relation to risk factors for coronary heart disease. *Journal of Clinical Pathology* **37**, 200-206

Erdman JW, Poor CL, Dietz JM (1988) Factors affecting the bioavailability of vitamin A, carotenoids and vitamin E. *Food Technology* **42**, 214-219

Fabjan N, Rode J, Kosir IJ, Wang ZH, Zhang Z, Kreft AI (2003) Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *Journal of Agricultural and Food Chemistry* **51**, 6452-6455

Faulks RM, Southon S (2001) Carotenoids, metabolism and disease. In: Wildman REC (Ed) *Handbook of Nutraceuticals and Functional Foods*, CRC Press LLC, Boca Raton, pp 143-156

Fogliano V, Vitaglione P (2005) Functional foods: Planning and development. *Molecular Nutrition and Food Research* **49**, 256-262

Gabrovska D, Fiedlerova V, Holasova M, Maskova E, Smrcinov H, Rysova J, Winterova R, Michalova A, Hutar M (2002) The nutritional evaluation of underutilized cereals and buckwheat. *Food and Nutrition Bulletin* **23**, 246S-249S

Gliszczyńska-Świgło A (2006) Antioxidant activity of water soluble vitamins in the TEAC (trolox equivalent antioxidant capacity) and the FRAP (ferrulic reducing antioxidant power) assays. *Food Chemistry* **96**, 131-136

Gordon M, Magos P (1983) The effect of sterols on the oxidation of edible oils.

Food Chemistry **10**, 141-147

Graf E, Eaton JW (1993) Suppression of colon cancer by dietary phytic acid. *Nutrition and Cancer* **19**, 11-19

Graf E, Empson KL, Eaton JW (1987) Phytic acid: A natural antioxidant. *The Journal of Biological Chemistry* **262**, 11647-11650

Gugliucci A, Menini T (2002) The botanical extracts of *Achyrocline satureoides* and *Ilex paraquariensis* prevent methylglyoxal-induced inhibition of plasminogen and antithrombin III. *Life Science* **72**, 279-292

Halliwell B, Murcia MA, Chirico S, Aruoma OI (1995) Free radicals and antioxidants in food and *in vivo*: what they do and how they work. *Critical Reviews in Food Science and Nutrition* **35**, 7-20

Harland BF, Oberleas D (1987) Phytate in foods. *World Review of Nutrition and Dietetics* **52**, 235-259

Hasenkopf K, Rönner B, Hiller H, Pischetsrieder M (2002) Analysis of glycated and ascorbylated proteins by gas chromatography-mass spectrometry. *Journal of Agricultural and Food Chemistry* **50**, 5697-5703

Hattori A, Migitaka H, Iigo M, Itoh M (1995) Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates. *Biochemistry and Molecular Biology International* **35**, 627-634

Herrmann K (1989) Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Critical Reviews in Food Science and Nutrition* **28**, 315-347

Hien TV, Huong NB, Hung PM, Duc NB (2002) Radioprotective effects of vitexin for breast cancer patients undergoing radiotherapy with cobalt-60. *Integrative Cancer Therapies* **1**, 38-34

Hofmann T (1998) Studies on the relationship between molecular weight and the color potency of fractions obtained by thermal treatment of glucose amino acid and glucose/protein solutions by using ultracentrifugation and color dilution techniques. *Journal of Agricultural and Food Chemistry* **46**, 3891-3895

Holasova M, Fiedlerowa V, Smrcinova H, Orsak M, Lachman J, Vavreinova S (2002) Buckwheat – the source of antioxidant activity in functional foods. *Food Research International* **35**, 207-211

Honke J, Zieliński H, Troszyńska A, Kozłowska H (2000) Inositol phosphates in raw and extruded cereals. *Czech Journal of Food Science* **18**, 251-253

Horbowicz M, Brenac P, Obendorf RL (1998) Fagopyritol B1, *O*-α-D-galactopyranosyl-(1→2)-D-*chiro*-inositol, a galactosyl cyclitol in maturing buckwheat seeds associated with desiccation tolerance. *Planta* **205**, 1-11

Horbowicz M, Obendorf RL (1992) Changes in sterols and fatty acids of buckwheat endosperm and embryo during seed development. *Journal of Agricultural and Food Chemistry* **40**, 745-750

Horbowicz M, Obendorf RL (2005) Fagopyritol accumulation and germination of buckwheat seeds matured at 15, 22, 30°C. *Crop Science* **45**, 1-11

Janet M, Horbowicz M, Obendorf RL (2005) Cyclitol galactosides in embryos of buckwheat steam-leaf-seed explants fed D-*chiro*-inositol, *myo*-inositol or D-pinitol. *Seed Science Research* **25**, 329-338

Jiang P, Burczynski F, Campbell C, Pierce G, Austria JA, Briggs CJ (2007) Rutin and flavonoid contents in three buckwheat species *Fagopyrum esculentum*, *F. tataricum*, and *F. homotropicum* and their protective effects against lipid peroxidation. *Food Research International* **40**, 356-364

Jones DP, Coates RJ, Flagg EW, Eley JW (1992) Glutathione in foods listed in the National Cancer Institute's Health Habits and History Food Frequency Questionnaire. *Nutrition and Cancer* **17**, 57-75

Kaiser S, Di-Mascio P, Murphy ME, Sies H (1990) Physical and chemical scavenging of singlet molecular by tocopherols. *Archives of Biochemistry and Biophysics* **277**, 101-108

Kawa JM, Taylor CG, Przybylski R (2003) Buckwheat concentrate reduces serum glucose in streptozotocin-diabetic rats. *Journal of Agricultural and Food Chemistry* **51**, 7287-7291

Kim AJ, Zaidul ISM, Maeda T, Suzuki T, Hashimoto N, Takigawa S, Noda T, Matsuura-Endo C, Yamauchi H (2007) A time-course of flavonoids in the sprouts of tartary (*Fagopyrum tataricum* Gaertn.) buckwheats. *Scientia Horticulturae* **115**, 13-18

Kim SL, Kim SK, Park CH (2004) Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. *Food Research International* **37**, 319-327

Kreft I, Fabjan N, Yasumoto K (2006) Rutin content in buckwheat (*Fagopyrum esculentum* Moench) food materials and products. *Food Chemistry* **98**, 508-512

Kris-Etherton PM, Hecker KD, Bonamone A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD (2002) Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *American Journal of Medicine* **113 (Suppl. 9B)**, 71-88

Krkošková B, Mrazova Z (2005) Prophylactic components of buckwheat. *Food Research International* **38**, 561-568

Lasztity R (1998) Oat grain - a wonderful reservoir of natural nutrients and biologically active substances. *Food Reviews International* **14**, 99-119

Lee GY, Jang DS, Lee YM, Kim JM, Kim JS (2006) Naphthopyrone glucosides from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation. *Archives of Pharmacal Research* **29**, 587-590

Lehtinen P, Laakso S (1997) Antioxidative-like effect of different cereals and cereal fractions in aqueous suspension. *Journal of Agricultural and Food Chemistry* **45**, 4606-4611

Leibovitz BE, Mueller JA (1993) Bioflavonoids and polyphenols: medical applications. *Journal of Optimal Nutrition* **2**, 17-35

Li D, Xiao G, Ding X (2000) Study on the free radical scavenging effects of tartary buckwheat flavone. *Food Science and Technology* **6**, 62-64

Li S, Zhang Q (2001) Advances in the development of functional foods from buckwheat. *Critical Reviews in Food Science and Nutrition* **41**, 451-464

Li W, Bollecker SS, Schofield JD (2005) Glutathione and related thiol compounds. I. Glutathione and related thiol compounds in flour. *Journal of Cereal Science* **39**, 205-212

Lintschinger J, Fuchs N, Moser H, Jäger R, Hlbeina T, Markolin G, Gössler W (1997) Uptake of various trace elements during germination of wheat, buckwheat and quinoa. *Plant Foods for Human Nutrition* **50**, 223-237

Liu RH (2004) Potential synergy of phytochemicals in cancer prevention: mechanism of action. *Journal of Nutrition* **134**, 3479S-3485S

Livrea MA, Luisa T, D'Arpa D, Morreale M (1997) Reaction of melatonin with lipoperoxyl radicals in phospholipid bilayers. *Free Radical Biology and Medicine* **23**, 706-711

Manach C, Scalbert A, Morand C, Remesey C, Jiménez L (2004) Polyphenols: food sources and bioavailability. *American Journal of Clinical Nutrition* **79**, 727-747

McKeehen JD, Busch RH, Fulcher RG (1999) Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to *Fusarium* resistance. *Journal of Agricultural and Food Chemistry* **47**, 1476-1482

Miglio C, Chiavaro E, Visconti A, Fogliano V, Pellegrini N (2008) Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry* **56**, 139-147

Mikulajova A, Takacsova M, Rapta P, Brindzova L, Zalibera M, Nemeth K (2007) Total phenolic contents and antioxidant capacities of cereal and pseudocereal genotypes. *Journal of Food and Nutrition Research* **46**, 150-157

Miller NJ, Rice-Evans CA (1996) Spectrophotometric determination of antioxidant activity. *Redox Report* **2**, 161-171

Murch SJ, Simmons CB, Saxena PK (1997) Melatonin in feverfew and other medicinal plants. *Lancet* **350**, 1598

Nicoli MC, Anese M, Parpinel M (1999) Influence of processing on the antioxidant properties of fruits and vegetables. *Trends in Food Science and Technology* **10**, 94-100

Normen L, Bryngelsson S, Johnsson M, Evheden LE, Brants H, Andersson H, Dutta P (2002) The phytosterol content of some cereal foods commonly consumed in Sweden and in the Netherlands. *Journal of Food Composition and Analysis* **15**, 693-704

Oomah DB, Mazza G (1996) Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry* **44**, 1746-1750

Ortmeyer HK, Huang IC, Zhang I, Hansen BC (1993) *Chiro*-inositol deficiency and insulin resistance. II. Acute effects of D-*chiro*-inositol administration in streptozotocin-diabetic rats, normal rats given a glucose load, and spontaneously insulin-resistant rhesus monkey. *Endocrinology* **132**, 646-651

Ortmeyer HK, Larner J, Hansen BC (1995) Effects of D-*chiro*-inositol added to a meal on plasma glucose and insulin in hyperinsulinemic rhesus monkeys. *Obesity Research* **3**, 605S-608S

Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F (2003) Total antioxidant capacity of plant foods, beverages, and oils consumed in Italy assessed by three different *in vitro* assays. *Journal of Nutrition* **133**, 2812-2819

Peng X, Zheng Z, Cheng K-W, Shan F, Ren G-X, Chen F, Wang M (2008) Inhibitory effect of mung bean extract and its constituents vitexin and isovitexin on the formation of advanced glycation endproducts. *Food Chemistry* **106**, 475-481

Peterson DM, Qureshi AA (1993) Genotype and environment effects on barley and oats. *Cereal Chemistry* **70**, 157-162

Picerno P, Mencherini T, Lauro MR, Barbato F, Aquino R (2003) Phenolic constituents and antioxidant properties of *Xanthosoma violaceum* leaves. *Journal of Agricultural and Food Chemistry* **51**, 6423-6428

Pietta P-G (2000) Flavonoids as antioxidants. *Journal of Natural Products* **63**, 1035-1042

Poeggeler B, Reiter RJ, Hardeland R, Sewerinek E (1995) Melatonin, a mediator of electron transfer and repair reactions, acts synergistically with the chain-breaking antioxidants ascorbate, trolox and glutathione. *Neuroendocrinology Letters* **17**, 87-92

Prabhakar MC, Bamo H, Kumar I, Shamsi MA, Khan SY (1981) Pharmacological investigations on vitexin. *Planta Medica* **43**, 396-403

Prior R, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry* **53**, 4290-4302

Przybylski R, Lee YC, Eskin NAM (1998) Antioxidant and radical-scavenging activities of buckwheat seed components. *Journal of the American Oil Chemists' Society* **75**, 1595-1601

Quettier-Deleu C, Gressier B,Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin J-C, Bailleul F, Trotin F (2000) Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of Ethnopharmacology* **72**, 35-42

Ramos S, Moulay L, Granado-Serrano AB, Vilanova O, Muguerza B, Goya L, Bravo L (2008) Hypolipidemic effect in cholesterol-fed rats of a soluble fiber-rich product obtained from cocoa husks. *Journal of Agricultural and Food Chemistry* **56**, 6985-6993

Rapała-Kozik M, Chernikevich IP, Kozik A (1999) Ligand-protein interaction in plant seed thiamine-binding proteins. Binding of various thiamine analoques to the sepharose-immobilized buckwheat seed protein. *Journal of Protein Chemistry* **18**, 721-725

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical in Biology and Medicine* **26**, 1291-1237

Reiter RJ (1997) Antioxidant actions of melatonin. *Advances in Pharmacology* **38**, 103-117

Reiter RJ (1995) Functional pleiotropy of the neurohormone melatonin: Antioxidant protection and neuroendocrine regulation. *Frontiers in Neuroendocrinology* **16**, 383-415

Rice-Evans CA, Miller NM, Paganda G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical in Biology and Medicine* **20**, 933-956

Rice-Evans CA, Miller NJ, Paganga G (1997) Antioxidant properties of phenolic compounds. *Trends in Plant Science* **2**, 152-159

Rice-Evans CA, Miller NJ (1994) Total antioxidant status in plasma and body fluids. *Methods in Enzymology* **234**, 279-293

Saastamoinen M, Plaami S, Kumpulainen J (1992) β-glucan and phytic acid content of oats cultivated in Finland. *Acta Agriculturae Scandinavica. Section B, Soil and Plant Science* **42**, 6-11

Sanchez-Moreno C, Cao G, Ou B, Prior RL (2003) Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with nontraditional wines obtained from high bush blueberry. *Journal of Agricultural and Food Chemistry* **51**, 4889-4896

Sandberg AS, Carlsson NG, Svanberg U (1989) Effects of inositol tri-, tetra-, penta-, and hexaphosphates on *in vitro* estimation of iron availability. *Journal of Food Science* **54**, 159-161

Saura-Calixto F (1998) Antioxidant dietary fiber product: A new concept and a potential food ingredient. *Journal of Agricultural and Food Chemistry* **46**, 4303-4306

Scalbert A, Johnson IT, Saltmarsh M (2005) Polyphenols: antioxidants and beyond. *American Journal of Clinical Nutrition* **81**, 215S-217S

Sensoy I, Rosen RT, Ho Ch-T, Karwe MV (2005) Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry* **99**, 388-393

Serafini M (2006) Back to the origin of the antioxidant hypothesis: the lost role of the antioxidant network in disease prevention. *Journal of the Science of Food and Agriculture* **86**, 1989-1991

Serafini M, Bellocco R, Wolk A, Ekstrom AM (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology* **123**, 985-991

Serrano J, Goni I, Saura-Calixto F (2007) Food antioxidant capacity determined by chemical methods may underestimate the physiological antioxidant capacity. *Food Research International* **40**, 15-21

Shamsuddin AM (1995) Inositol phosphates have novel anticancer function. *Journal of Nutrition* **125**, 725-732

Shamsuddin AM, Elsayed AM, Ullah A (1988) Suppression of large intestinal cancer in F344 rats by inositol hexaphosphate. *Carcinogenesis* **9**, 577-582

Shevchuk TE (1983) The content of rutin in different sorts of buckwheat. *Fagopyrum* **3**, 12-19

Shida CS, Castrucci AML, Larny-Freund MT (1994) High melatonin solubility in aqueous medium. *Journal of Pineal Research* **16**, 198-201

Singh RP, Sharma G, Mallikarjuna GU, Dhanalakshmi S, Agarwal C, Agarwal R (2004) *In vivo* suppression of hormonerefractory prostate cancer growth by inositol hexaphosphate: induction of insulin-like growth factor binding protein-3 and inhibition of vascular endothelial growth factor. *Clinical Cancer Research* **10**, 244-249

Slavin JL (2000) Mechanisms for the impact of whole grain foods on cancer risk. *Journal of the American College of Nutrition* **19**, 300S-307S

Somasundar PS, Riggs DR, Jackson BJ, Cunningham C, Vona-Davis L, McFadden DW (2004) Inositol hexaphosphate (IP6): A novel treatment for pancreatic cancer. *Journal of Surgical Research* **126**, 199-203

Stanner SA, Hughes J, Kelly CN, Buttriss J (2004) A review of the epidemiological evidence for the 'antioxidant hypothesis'. *Public Health Nutrition* 7, 407-422

Steadman KJ, Burgoon MS, Lewis BA (2001a) Buckwheat seed milling fraction: description, macronutrients composition and dietary fibre. *Journal of Cereal Science* **33**, 271-278

Steadman KJ, Burgoon MS, Lewis BA, Edwardson SE, Obendorf RL (2001b) Minerals, phytic acid, tannin and rutin in buckwheat seed milling fractions. *Journal of the Science of Food and Agriculture* **81**, 1094-1100

Steadman KJ, Burgoon MS, Seuster RL, Lewis BA, Edwardson SE, Obendorf RL (2000) Fagopyritols, D-*chiro*-inositol, and other soluble carbohydrates in buckwheat seed milling fractions. *Journal of Agricultural and Food Chemistry* **48**, 2843-2847

Sugiyama K, Kushima YD, Muramatu K (1985) Effects of sulfur-containing amino acids and glycine on plasma cholesterol diet. *Agricultural and Biological Chemistry* **49**, 3455-3461

Tantivejkul K, Vucenik I, Shamsuddin AM (2003) Inositol hexaphosphate (IP6) inhibits key events of cancer metastasis: II. Effects on integrins and focal adhesions. *Anticancer Research* **23**, 3681-3689

Thompson LU (1993) Potential health benefits and problems associated with antinutrients in food. *Food Research International* **26**, 131-149

Vakkuri O, Leppaluto J, Kauppila A (1985) Oral administration and distribution of melatonin in human serum, saliva and urine. *Life Science* **37**, 489-495

Valtuena S, Pellegrini N, Franzini L, Bianchi MA, Ardigo D, Del Rio D, Piatti P, Scazzina F, Zavaroni I, Brighenti F (2008) Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. *American Journal of Clinical Nutrition* **87**, 1290-1297

Ververidis F, Trantas E, Douglas C, Vollmer G, Kretzschmar G, Panopoulos N (2007) Biotechnology of flavonoids and other phenylpropanoid-derived natural products. Part I: Chemical diversity, impacts on plant biology and human health. *Biotechnology Journal* **2**, 346-387

Vitaglione P, Napolitano A, Fogliano V (2008) Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology* **19**, 451-463

Watanabe M (1998) Catechins as antioxidants from buckwheat (*Fagopyrum esculentum* Moench) groats. *Journal of Agricultural and Food Chemistry* **46**, 839-845

Watanabe M (2007) An anthocyanin compound in buckwheat sprouts and its contribution to antioxidant capacity. *Bioscience, Biotechnology, and Biochemistry* **71**, 579-582

Wijngaard HH, Arendt EK (2006) Buckwheat. *Cereal Chemistry* **83**, 391-401

Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II Review of 93 intervention studies. *American Journal of Clinical Nutrition* **81**, 243S-255S

Wolf KL, Kang X, He X, Dong M, Zhang Q, Liu RH (2008) Cellular antioxidant activity of common fruits. *Journal of Agricultural and Food Chemistry* **56**, 8418-8426

Xuan TD, Tsuzuki E (2004) Allelopathic plants: Buckwheat (*Fagopyrum* sp.). *Allelopathy Journal* **13**, 137-148

Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H (2000) Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *Journal of Agricultural and Food Chemistry* **48**, 180-185

Yao Y, Shan F, Bian J, Chen F, Wang M, Ren G (2008) D-*chiro*-inositol-enriched tartary buckwheat bran extracts lowers the blood glucose level in KK-Ay mice. *Journal of Agricultural and Food Chemistry* **56**, 10027-10031

Yasuda T, Masaki K, Kashiwagi T (1992) An enzyme degrading rutin in tartary buckwheat seeds. *Journal of the Japanese Society for Food Science and Technology* **39**, 994-1000

Zhang Y, Bao B, Lu B, Ren Y, Tie X, Zhang Y (2005) Determination of flavone *C*-glucosides in antioxidant of bamboo leaves (AOB) fortified foods by reversed-phase high-performance liquid chromatography with ultraviolet diode array detection. *Journal of Chromatography A* **1065**, 177-185

Zhang Y, Jiao J, Liu C, Wu X, Zhang Y (2008) Isolation and purification of four flavone *C*-glycosides from antioxidant of bamboo leaves by macroporous resin column chromatography and preparative high-performance liquid chromatography. *Food Chemistry* **107**, 1326-1336

Zielińska D, Szawara-Nowak D, Zieliński H (2007a) Comparison of spectrophotometric and electrochemical methods for the evaluation of antioxidant capacity of buckwheat products after hydrothermal treatment. *Journal of Agricultural and Food Chemistry* **55**, 6124-6131

Zielińska D, Szawara-Nowak D, Ornatowska A, Wiczkowski W (2007b) The use of cyclic voltammetry, photochemiluminescence and spectrophotometric methods for the measurement of the antioxidant capacity of buckwheat sprouts. *Journal of Agricultural and Food Chemistry* **55**, 9891-9898

Zieliński H, Michalska A, Piskuła MK, Kozłowska H (2006) Antioxidants in thermally treated buckwheat groats. *Molecular Nutrition and Food Research* **50**, 824-832

Zieliński H, Kozłowska H, Lewczuk B (2001) Bioactive compounds in the cereal grains before and after hydrothermal processing. *Innovative Food Science and Emerging Technologies* **2/3**, 159-169