REVIEW

Antioxidant Properties of Whole Grain Cereals

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Summary

Cereals have a long history of use by humans. Cereals and cereal products are staple foods, and are important source of energy, carbohydrate, protein, fibre, vitamins (E, B) and minerals (Zn, Mg, Fe) in both developed and developing countries. The health aspects of whole grain cereals have long been known, but the antioxidant profile of whole grains has only recently been introduced to the antioxidant research community where mostly fruits and vegetables are in focus. In vitro experiments confirm antioxidant richness of whole grains and their specific fractions, but further in vivo investigations are needed to confirm the activity. Great variety of whole grain antioxidants suggest that regular consumption of whole grain cereals may have a significant role in slowing down degenerative processes and prevention of chronic diseases.

Key words: whole grain cereals, antioxidants, antioxidant capacity, health effect

Sažetak

Uporaba žitarica seže daleko u ljudsku povijest. Žitarice i proizvodi od žitarica su osnova svakodnevne prehrane i glavni su izvor energije, ugljikohidrata, proteina, vlakana, vitamina (E, B) i minerala (Zn, Mg, Fe) kako u razvijenim, tako i u zemljama u razvoju. Učinak koji imaju cjelovite žitarice na zdravlje je već dugo poznat, no tek su u zadnje vrijeme, uz antioksidanse voća i povrća koji se uglavnom nalaze u fokusu istraživanja, brojna istraživanja usmjerena i na određivanje anioksidativnih svojstava žitarica. Pokusi in vitro potvrdili su kako su cjelovite žitarice i pojedini dijelovi zrna bogati antioksidansima, ali su potrebna daljnja in vivo ispitivanja koja bi potvrdila njihovo djelovanje. Velika raznolikost antioksidansa u cijelom zrnu žitarica daje naslutiti da bi redoviti unos integralnih žitarica mogao imati značajnu ulogu u usporavanju degenerativnih procesa u organizmu i prevenciji raznih kroničnih oboljenja.

Ključne riječi: integralne žitarice, antioksidansi, antioksidativni kapacitet, zdravstveni učinak

Introduction

Whole grain cereals have gained popularity and been introduced to healthy eating trends because of their potential protection against age-related diseases such as diabetes, cardiovascular diseases, some cancers and all-cause mortality (Fardet et al., 2008). This effect on the body is thought to be conducted through few mechanisms (e.g. large-bowel effects of whole grains; glucose and insulin changes; the antioxidant theory for whole grain protectiveness; phytoestrogen activity) and can be linked to the variety of their fibre, phytochemical and micronutrient composition (Slavin, 2003). All of these potential protective mechanism are in focus of many scientists, but recent research has shown that the total phytochemical content and antioxidant activity of the whole grains have been underestimated in the literature, and that the whole grains contain more phytochemicals than was previously reported (Liu, 2007). When talking about foods antioxidant activity, this potential has been usually linked to fruits and vegetables, but it is more and more believed that the whole grain cereals could play a great role in contributing to daily antioxidant intake. This review presents the notable researches on *in vitro* and *in vivo* whole grain antioxidant effect done in the last years.

ANTIOXIDANTS IN WHOLE GRAINS

Nutritional status is an important factor contributing to health promotion and/or disease prevention. Nutrients that have been shown to have positive effect on health include different types of vitamins and minerals (such as zinc, copper and selenium), but there is an increasing amount of data showing the significance of the phytochemicals. Whole grains are rich in a wide range of phytochemicals which have been recognized to support overall health through their antioxidant potential (Figure 1). The primary protective function of antioxidants in the body is the reaction with free radicals. Whole grain antioxidants are thought to act as direct free radical scavengers, cofactors of antioxidant enzymes or as indirect antioxidants (Fardet et al., 2008). Free radical compounds result from normal metabolic activity as well as from the diet and environment but can cause oxidative damage to large biomolecules such as

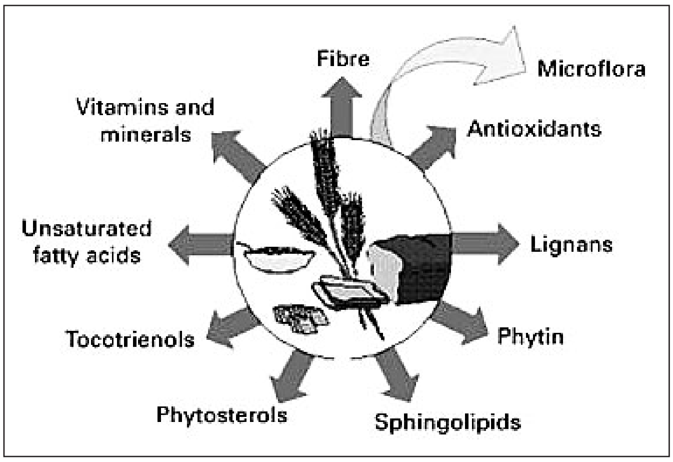

Figure 1. *Phytonutrients of the whole grain cereals (Slavin, 2003)*

DNA, lipids and protein, resulting in an increased risk of several chronic diseases and contributing to general inflammatory response and tissue damage (Slavin, 2003).

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It is recognised that reactive oxygen species (ROS) are involved in a variety of physiological and pathological processes, including cellular signal transduction, cell proliferation, differentiation and apoptosis, as well as ischemia – reperfusion, inflammation, and many neurodegenerative disorders. In healthy individuals ROS production is continuously balanced by natural antioxidative defence systems. A process where the physiological balance between pro-oxidants and antioxidants is disrupted in favour of the former, resulting in potential damage for the organism, is called oxidative stress (Qingming et al., 2010). Thus, it follows that if dietary antioxidants reduce free radical activity in the body, then disease potential is reduced. Dietary antioxidants of whole grain cereals are mostly located in the outer and the bran fractions of grains (folates, phenolic acids, carotenoids, betaine, choline, sulphur amino acids, phytic acid, lignans, avenanthramides, β-glucan and alkylresorcinols) or as in case of vitamin E, in the germ. Different antioxidant mechanisms have been attributed to whole grain phytonutrients, such as prevention of the oxidation of polyunsaturated lipids (vitamin E), reduction of the concentration of plasma homocysteine (vitamin B9, betaine, choline), acting as cofactors of antioxidant enzymes superoxide dismutase, glutathione peroxidase and thioredoxine reductase (Zn, Fe, Se, Cu, Mn) or stabilization and delocalization of unpaired electrons (vitamin E, polyphenols, alkylresorcinols). Phenolic acids (ferulic, vanillic, syringic, sinapic and *p*-coumaric acid) are also believed to chelate transition metals as well as to activate or repress particular genes, while sulphur amino acids (cysteine, methionine) contribute to the synthesis of a major endogenous antioxidant gluthatione (Fardet et al., 2008). Whole grain antioxidants are water- and fat- soluble, and approximately half are insoluble. A majority of the insoluble antioxidants are bound as cinnamic acid esters to arabinoxylan side chains of hemicellulose. Covalently-bound phenolic acids are good free radical scavengers. It is considered that antioxidants found in wholegrain foods provide protection over a long time period through the entire digestive tract (Slavin, 2003).

IN VITRO WHOLE GRAIN ANTIOXIDANT ACTIVITY

Objective of the *in vitro* assays is to predict the biological efficiency of antioxidants and the method is considered to be appropriate if the results correlate well with the *in vivo* antioxidant capacity. Most of the methods used for evaluation of antioxidant capacity of different cereals, their specific fractions or products are based on two types of chemical reactions involved: Hydrogen Atom Transfer (HAT) reactions and single Electron Transfer (ET) reactions. The majority of HAT based assays apply a competitive reaction scheme in which antioxidant and substrate compete for thermally generated peroxyl radicals through the decomposition of azo compounds. These assays include inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assays. ET based assays measure the capacity of an antioxidant by the reduction of an oxidant, which changes color when reduced. The degree of color change is correlated with the sample's antioxidant concentrations. These assays include the total phenols assay by Folin Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC), ferric ion reducing antioxidant power (FRAP), "total antioxidant potential" assay using a Cu(II) complex as an oxidant, and 2,2-Diphenyl-1-Picrylhydrazyl method (DPPH). Other assays measure a sample's scavenging capacity of biologically relevant oxidants such as single oxygen, superoxide anion, peroxynitrite, and hydroxyl radical (Huang et al., 2005).

Adom and Liu (2002) used total oxyradical scavenging capacity (TOSC) assay to investigate soluble, conjugated and insoluble bound forms of phytochemicals in uncooked wheat, oats, corn and rice as well as their antioxidant activity. The major portion of phenolics was found to exist in the bound form - more than 60% depending on the cereal. Corn had the highest phenolic content, followed by wheat, oats and rice. Antioxidant activity showed to be positively correlated with phenolic content, thus corn had the highest and rice lowest activity. Bound compounds were the major contributors to the total antioxidant activity. Another study using TEAC (Martinez-Tome et al., 2004) confirmed weaker oat bran antioxidant activity compared to different wheat bran products. Phenolic acid composition, tocopherol content, carotenoid profile and total phenolic content were examined for determination of the phytochemical composition of wheat bran and related antioxidant activity (Zhou et al., 2004). The data suggests that wheat and wheat bran from different countries may differ in their antioxidant potentials. Less common grains, such as sorghum have also been analyzed for antioxidant activity and phenolic content (Awika et al., 2003). Phenolic content of different cultivars of sorghums, sorghum bran, baked and extruded products correlated highly with their antioxidant activity. Esposito et al. (2005) showed that the antioxidant activity of durum wheat differs in three bran layers and it increases in fractions with reduced granulometry. The antioxidant activity of some durum wheat by-product fractions measured by ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) is comparable to that of well know antioxidant foodstuff such as red wine, tomatoes or peaches, likely due to the presence of fibre-bound phenol compounds. Liu and Yao (2007) examined extraction techniques of antioxidants from barley and proved

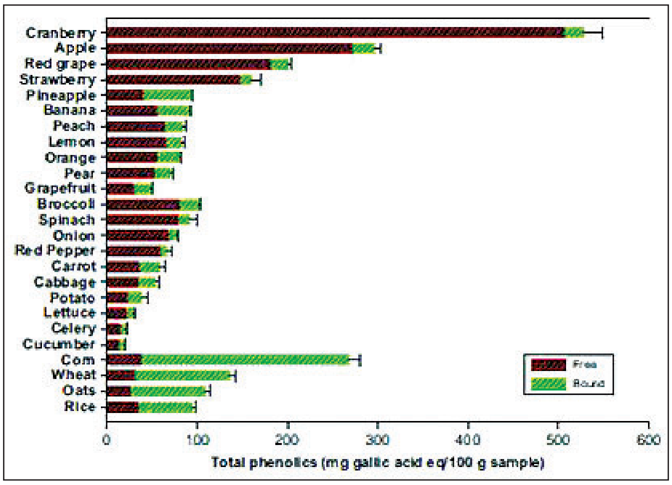

Figure 2. *Total phenolic content of common fruits, vegetables and whole grains (Liu, 2007)*

Table 1. *Antioxidant content of the whole grain cereals (Fardet et al., 2008)*

Cereal type (variety)	Tocopherol and tocotrienol (mg/100 g grain)	Folates (mg/100 g grain)	Zinc (mg/100 g grain)	Iron (mg/100 g grain)	Copper (mg/100 g grain)	Selenium (mg/100 g grain)	Manganese (mg/100 g grain)	Polyphenols (mg gallic acid eq./ 100 g grain)
Wheat	1.4	87	2.6	3.2	369	0.5-74.6	3.1	70-1459
Maize (yellow, white & blue)	6.6	110-170	1.7	1.5	240	12.0	0.4	39-711
Rice (brown & black	1.9	16	1.6	3.2	288	10.0	2.1	54-313
Oats	1.8	33	3.2	5.8	422	7.1	3.1	9-34
Barley	2.2	65	2.8-7.4	2.8-12.8	419-570	7.0	1.5	50-196
Rye	4.1	143	2.9-3.1	2.8-4.4	290-392	1.4	2.9	125-255
Sorghum (white, yellow, red & brown)	1.1	-	0.3-1.8	1.1-5.7	20-735	13.0	4.3	100-2300
Millet (shucked grain)	4.0	-	2.9-6.6	6.9-20	340-610	2.0	1.1	29-47
	Ferulic acid (mg/100 g grain)	**Betaine (mg/100 g)**	**Choline (mg/100 g)**	**Phytic acid (mg/100 g grain)**	**Lignin (g/100 g grain)**	**Lignan (mg/100 g grain)**	**Carotenoids (mg/100 g grain)**	**Alkylresorcinols (mg/100 g grain)**
Wheat	10-198	6.9 (flour & germ)	1.6 (flour & germ)	906	0.6-1.3	490	20-265	28-142
Maize (yellow, white & blue)	177	-	-	940	-	-	969-1300	-
Rice (brown & black	30	0.5 (brown)	-	890	-	-	14-77	0
Oats	7-30	2.7 (plain)	1.3 (plain)	900	-	-	31	-
Barley	36-62	-	-	1070	-	-	15-105	4-44
Rye	79-102	-	-	970	-	95	-	31-278
Sorghum (white, yellow, red & brown)	9	-	-	-	-	-	20-22	0
Millet (shucked grain)	29	-	-	-	-	-	74-80	9-87

that the properties of the extracting solvents significantly affected the total phenolics, proanthocyanidins and antioxidant activities of barley extract. Miller et al. (2000) used the DPPH assay to show that the average cereals and cereal products antioxidant activity (measured as Trolox equivalents) is higher (between 1200 and 3500 mmol TE/100 g of fresh product) than that of common fruit (1200 mmol TE/100 g) and vegetables (400 mmol TE/100 g), but lower than that of common berries (around 3880 mmol TE/100 g). The antioxidant capacity of the wheat bran fraction was 8500 mmol TE/100 g and that of the germ fraction was 5000 mmol TE/100 g. Thus, whole grain cereals have higher antioxidant capacity than some of the most common fruits and vegetables (Figure 2). The results of the researches on antioxidant content of the whole grain cereals presented in Table 1 suggest that the antioxidant activity of cereal products is far from negligible.

IN VIVO WHOLE GRAIN ANTIOXIDANT ACTIVITY

Although in vitro experiments indicate great antioxidant abilities of the whole grain cereals, it is questionable if chemical methods underestimate physiological antioxidant capacity. Several studies have been done in order to evaluate bioactivity of whole grain phytochemicals, mostly on animals.

To determine the protective effects of oxidative damage to DNA by supplementation with rice bran oil, the rats with streptozotocin induced diabetes were fed with rice bran oil, rich in different phytochemicals (γ-oryzanol, γ-oryzanol, α-tocopherol) and with more than 85% of PUFA and MUFA. By measuring the levels of 8-OhdG (used as a sensitive biomarker of oxidative DNA damage and oxidative stress) oxidative DNA damage in various tissues of streptozotocin induced diabetic rats was examined. Significant reductions in mtDNA 8-OhdG levels were seen in the liver, kidney, and pancreas of diabetic rats treated with rice bran oil compared with diabetic rats without intervention (Hsieh et al., 2005). Similar, rats fed with fructose diet for 4 weeks were evaluated for their hyperlipidemia and insulin resistance after they were given anthocyanin-rich extract from black rice. Fructose fed rats exhibited significantly higher plasma insulin levels and lower insulin sensitivity than the control rats. Dietary supplementation with the anthocyanin-rich extract prevented the development of fructose-induced insulin resistance showing potential property of preventing metabolic syndrome by improving lipid profile and increasing insulin sensitivity in fructose-fed rats (Guo et al., 2007). The study on investigation whether black rice extract (BRE) supplementation might ameliorate oxidative stress and enhance antioxidant enzyme activities in HepG2 cells and in C57BL/6 mice in the cellular study showed significant suppression of superoxide anions ($O_2^{\bullet-}$) and reactive oxygen species (ROS) in the BRE group. The BRE group also showed significant increases in superoxide dismutase (SOD) and catalase (CAT) activities. In the animal study plasma HDL-cholesterol was significantly higher, and thiobarbituric, acid-reactive substances were significantly lower in the BRE group, whereas plasma levels of total cholesterol and triglyceride were not affected by BRE supplementation. Increased hepatic SOD and CAT activities were observed in BRE-treated mice as compared to the control mice. However, no changes were detected for the protein expression of antioxidant enzymes (Chiang et al., 2006). *In vivo* effect of absorbed oat avenanthramides and phenolic acids on the antioxidant capacity of hamsters has been tested by Chen et al. (2004). Absorbed oat phenolics did not change the resistance of hamster LDL against Cu^{2+}-induced oxidation until ascorbic acid was added to the assay mixture. Álvarez et al. (2006) fed healthy mice for 5 weeks with different types of cereal fractions (wheat germ, buckwheat flour, rice bran and wheat middlings) rich in polyphenols. The amount of total phenolic compounds in all extracts was determined by Folin Ciocalteu method and the antioxidant activity of the selected cereal product extracts evaluated using an ORAC procedure. Effect on some parameters of function and redox state of peritoneal leukocytes were measured (lymphocyte chemotaxis capacity, microbicidal activity, lymphoproliferative response to mitogens, interleukin-2 and tumor necrosis factor release, as well as oxidized glutathione, catalase activity and lipid oxidative damage). All cereal fractions caused an improvement/increase of the leukocyte parameters studied. In alloxane induced diabetic rats, fed with millet diet over a period of 28 days, blood glucose, cholesterol, enzymatic and nonenzymatic antioxidants, lipid peroxides in blood plasma, and glycation of tail tendon collagen were measured. The levels of enzymatic (glutathione, vitamins E and C) and nonenzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and lipid peroxides were significantly reduced in diabetic animals and restored to normal levels in the millet-fed groups. There was a significant lowering of blood sugar and total cholesterol in rats fed diets formulated with millet, but difference between different varieties of millet was observed (Hegde et al., 2005). Qingming et al. (2010) determined the antioxidant properties of malt extract *in vivo*. When given to D-galactose-treated mice, the malt extract from barley exhibited high antioxidant activities. That was evidenced by its ability to scavenge hydroxyl- and superoxide- radicals, high reducing power and protection against biological macromolecular (lipid, protein, DNA) oxidative damage. Malt extract prevented the decrease of antioxidant enzyme activities, decreased liver and brain malondialodehyde levels and carbonyl content, and improved total antioxidant capability in mice.

Human intervention studies on antioxidant potential of cereals have been studied to a much lesser extent compared to animal and in vitro studies. Research conducted on 76 male patients with Coronary Artery Disease during 16 weeks showed that replacement of refined rice with whole grain powder resulted in substantial improvement in glucose and insulin metabolism as well as reduction in lipid peroxidation and plasma homocysteine concentration (Jang et al., 2001). When insulin sensitivity and markers of lipid peroxidation and inflammation were evaluated on healthy, moderately overweight subjects (22 F, 8 M), there was no difference observed between two periods (of 6 week) of given refined and whole grain diet although the previous study on CAD patients showed reduced markers after the consumption of wholegrain products (Andersson et al., 2007). Another study on 12 middle-aged hypercholesterolemic women who were fed refined and phytochemical-rich diet (including fruits, vegetables and tea) showed decrease in the oxidative enzymes - erythrocyte superoxide dismutase and plasma glutathione peroxidase (Bruce, 2000). Study on bioavailability of oat avenanthramides on 6 older adults, showed increased antioxidant capacity (by measuring plasma reduced glutathione and oxidized glutathione) after consumption of milk enriched with avenanthramides (Chen et al., 2007). The potential antioxidative effect of the ferulic acid from rye bran was investigated by measuring LDL susceptibility to copper oxidation *ex vivo* in 18 healthy postmenopausal women, but within 12 weeks of study the elevated ferulic acid had no influence on lag time or propagation rate of the LDL oxidation (Harder et al., 2004). Another study included sixty adult patients with coronary heart disease, supplemented with black rice pigment fraction for 6 months. Compared to white rice pigment fraction supplemented control group, the black rice pigment subjects showed greatly enhanced plasma total antioxidant capacity (Wang et al., 2007).

FUTURE PERSPECTIVES

Although there are enough amounts of *in vitro* experiments confirming potential antioxidant richness of whole grains and their specific fractions, there is still lack of in vivo experiments to confirm the results of former. Additionally, results from both types of experiments are difficult to compare since the methods for evaluation of antioxidant capacity differ and are not standardized.

There are several kinds of problems when dealing with whole grains as an antioxidant source. First problem is linked to the extraction efficiency of specific compounds. Since whole grains are solid material, extraction protocols can be insufficient for target compound isolation. Most studies have reported the phenolic levels of grains using various aqueous solutions of methanol, ethanol and acetone to extract soluble phenolics. These methodologies assumed that long extraction times and/or use of finely powdered samples would ensure maximum extraction of phenolic compounds from grains. However, in this step it should be taken into account nature and matrix position of grain phytochemicals, their structure and bonds with other cell wall materials, such as the arabinoxylan, proteins, cellulose and lignin polymers (Liu, 2007). It is considered that more than 60% of phenolics are in bound form (Slavin, 2003), although there is still limited literature on the complete spectrum and of grain phenolic compounds and linkages to the other matrix components. Most *in vitro* experiments confirm whole grain antioxidant activity, but it is uncertain if the amounts of specific phytochemicals accurately reflect the amount present in plasma and tissues, since their metabolism pathway can cause loss of antioxidant action or their concentration is not enough to act as significant free radical scavengers.

In vivo experiments have been done both on animals and humans, with animal studies showing more consistent results, confirming grain antioxidant hypothesis. Despite, they are hardly comparable since they include different types of grains and examine different aspects of antioxidant activity. Few human intervention studies indicate that the whole grains could contribute to health from antioxidant point of view as well as through other already known mechanism. Results of human studies vary, showing both positive and no effect of grains on oxidative stress. In some studies the partial antioxidant profile (meaning determination of few specific compounds) of given food was examined, but it cannot be concluded that only specific compounds contribute to the positive effect. Since the theory of synergistic effect of different food constituents on health is more and more likely to be true, more focus should be put on discovering complete antioxidant profile, as well as interactions among specific compounds, their metabolism and bioavailability in general. Because of the complex composition of the grain extracts, to evaluate the total antioxidant activity more methods should be employed in order to separate each compound and study it individually, which is costly and inefficient. Also, effect on food processing should be taken into consideration, as a strong factor affecting distribution, stability and activity of grain antioxidants. There is still plenty of work to be done in order to know the antioxidant capacity and constituents in the foods we consume. Primary focus should be on finding validated and convenient assay for the quick quantitation of antioxidant effectiveness. Although at the moment seems that only combination of numerous methods could achieve this goal, broadening the knowledge on this topic and development of the technology could lead to more efficient ways. For now, one solution could be one comprehensive study, including more teams who could cover all aspects of antioxidant research, from basics including extraction and chemical methods to complete human intervention studies.

CONCLUSION

Cereal grains comprise complex mixtures of components (folates, phenolic acids, carotenoids, betaine, choline, sulphur amino acids, phytic acid, lignans, avenanthramides, β-glucan and alkylresorcinols, vitamin E) which differ in their distributions between the various tissues and cell types. A high consumption of cereals has been associated with a decreased risk of developing several chronic diseases since they provide a range of macro- and micronutrients. Some of these have been known to act as antioxidants. While whole grain cereals are yet to be confirmed as an important source of antioxidants and exact mechanism by which cereals convey beneficial effects on health should be clarified, their potential antioxidant activity contributes to the good image grains gained in the last years which could finally result in increased whole grain intake among consumers.

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