

Introduction

Much has been written over the past 15 years regarding the potential “health benefits” associated with beans if they are included as a significant component of the diet. In the late 1980s, dry beans were touted as the “heart healthy” food for the 1990s. Although the “beans are good for the heart” campaigns of the 1990s have subsided a bit in the new millennium, beans should not be overlooked as an important food that can lower the risk of heart and other debilitating diseases and improve health and well being. Beans are a good source of several nutrient and non-nutrient plant compounds that have health promoting effects.^{1,2}

Potential Health Benefits from Antioxidant Compounds in Dry Beans

Over the past decade, many consumers have been making dietary choices based on natural chemicals, generally referred to as “phytochemicals,” present in foods. There are hundreds of phytochemicals in fruits and vegetables seen as beneficial to health because of their attributes as antioxidants. Nowadays, much attention is given to the effects of antioxidants in diets.

A common example of an antioxidant is vitamin C (ascorbic acid) found abundantly in citrus fruits. Vitamin E and the carotenoids—pigments that give carrots, tomatoes, and yellow fleshed fruits and vegetables their color—are effective antioxidants.³ Antioxidants protect cells and tissues from the damaging effects of certain highly reactive atoms or molecules called free radicals. A free radical is an atomic species with an unpaired electron. How free radicals form in the body and how antioxidants work to inhibit their effects will be discussed in depth later in the article.

Seed coats of colored dry beans are a rich source of a particular group of compounds with antioxidant activity. How are seed coat color and antioxidants related in beans? The color of beans is determined by the presence and amounts of pigments belonging to a group of phytochemicals known as flavonoids. The generalized structure of a flavonoid molecule is shown in Figure 1. Flavonoids are simple and complex phenolic

compounds that are found in a wide range of plants, and that perform a wide range of functions in plant tissues.⁴ The specific flavonoids found in bean seed coats are flavonols, anthocyanins, and condensed tannins (proanthocyanidins).⁵

Recently, scientists at the USDA-ARS Dry Bean Genetics Laboratory located on the campus of Michigan State University isolated and identified the particular flavonoids associated with colors in gray, brown, yellow, red, and black beans.⁶⁻¹¹ These colors were chosen because they distinguish several important market classes of beans recognized in the U.S. (e.g., the red, black, and brown seed coat colors of the kidney bean, small red, black, and pinto market classes). The various flavonoid compounds identified in seed coats of the

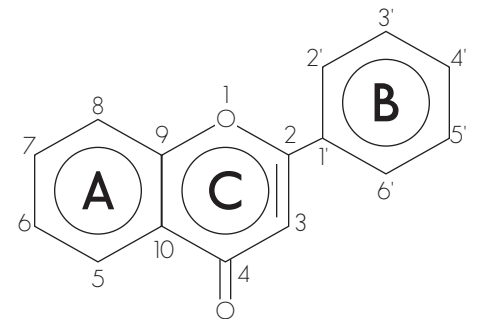


Figure 1. General structure of a flavonoid molecule. Flavonoids are 15 carbon cyclic compounds with two phenyl rings, A and B. The A-ring is comprised of six carbons of three acetate molecules while the B-ring and part of the C-ring carbons are from phenylalanine. The most important structural feature of flavonoids for antioxidant activity is the B-ring ortho 3', 4' dihydroxy orientation.

Table 1. Flavonoid Compounds Present in Seed Coats, Seed Coat Color, and Genotype Name for the Genetic Stocks Used in the Antioxidant Assay.

Seed coat color and genotype name	Flavonoid compounds present in seed coat*
Gray VOO59	Condensed tannins
Brown Buffy citrine Mineral brown Matte mineral brown	K3G, condensed tannins K3G, condensed tannins K3G, condensed tannins
Yellow Manteca Yellow brown	K3GX, K3G K3G, condensed tannins
Dark blue; black Fla. Breeding line 5-593 Matte black Dark brown violet	D3G, P3G, M3G, condensed tannins D3G, P3G, M3G, condensed tannins D3G, P3G, M3G, condensed tannins
Red Dark red kidney Light red kidney	Q3GX, Q3G, K3G, condensed tannins Q3G, condensed tannins

*K3G = kaempferol 3-O-glucoside; K3GX=kaempferol 3-O-glucose-xylose; D3G=delphinidin 3-O-glucoside; P3G = petunidin 3-O-glucoside; M3G=malvidin 3-O-glucoside; Q3G=quercetin 3-O-glucoside; Q3GX = quercetin 3-O-glucose-xylose

different colored beans are presented in Table 1. After the flavonoids were identified, they were purified from seed coat extracts and tested for their antioxidant efficacy.²

The antioxidant assay consisted of adding individual flavonoid compounds to liquid suspensions of very tiny fat bodies called liposomes.² Intact liposomes fluoresce and the amount of fluorescence was recorded with an instrument called a fluorometer. Oxidation of the liposome suspension caused the liposomes to break apart, which in turn, caused them to lose their fluorescing ability. The loss of fluorescence was compared to the commercial antioxidant, BHT (butylated hydroxytoluene), used as the control in the experiments.

BHT almost completely inhibited oxidation in the tests (Figure 2). The anthocyanins, delphinidin 3-O-glucoside and petunidin 3-O-glucoside and the flavonol, quercetin 3-O-glucoside were the most active of the pure compounds tested (Figure 2). The third anthocyanin—malvidin 3-O-glucoside—showed antioxidant activity

but was significantly less effective than BHT, delphinidin 3-O-glucoside, petunidin 3-O-glucoside, and quercetin 3-O-glucoside in preventing liposome destruction. Kaempferol 3-O-glucoside—a flavonol found in most beans tested except black—had the least antioxidant activity. Its activity was less than 20% relative to BHT. Tannins (phenolic polymers) also were found to have potent antioxidant activity.² This was not surprising because of the reactive hydroxyl groups characteristic of the flavonoid skeleton from which tannins are formed through polymerization.

Other research also has shown condensed and hydrolyzable tannins to be powerful antioxidants. For example, tannins from sorghum were found to be 15-30 times more effective than simple phenols at quenching free radicals.¹² Similarly, tannins isolated from adzuki bean were found to have antioxidant properties.¹³ Tannin containing extracts from a variety of dry beans inhibited iron-catalyzed oxidation of soybean oil.¹⁴

Since tannin chemistry is complicated and separation techniques to purify tannins are difficult and often give unreliable results, the antioxidant activity of tannins was restricted to those present in methanol extracts—the solvent used to dissolve and extract the pigments in the seed coats. In addition to the tannins, the methanol extracts also contained simple flavonoids (flavonoid monomers like quercetin). Tannins were found to have a higher antioxidant activity than the simple flavonoid compounds: delphinidin, petunidin, malvidin, quercetin, and kaempferol.²

Free Radicals

The cells of one's body are in a constant state of flux. Biochemical reactions are constantly occurring. The body uses its energy-yielding nutrients to fuel its metabolic and physical activities.³ Oxygen is a key element in the body's metabolism. Oxygen has eight protons (positively charged particles) in its nucleus and eight electrons (negatively charged particles) that travel in paths about the nucleus. The eight positive and eight negative charges of elemental oxygen cancel each other, thus, leaving the atom electrically neutral to its surroundings. Oxygen's eight electrons

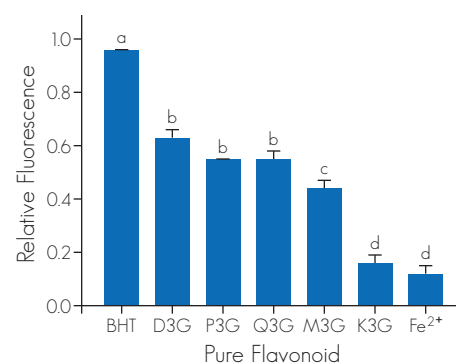


Figure 2. Antioxidant activity (1.0ug/ml) after 15 min relative to the control of pure flavonoids isolated and identified from dry bean seed coats. BHT, butylated hydroxytoluene; D3G, delphinidin 3-O-glucoside; P3G, petunidin 3-O-glucoside; Q3G, quercetin 3-O-glucoside; M3G, malvidin 3-O-glucoside; K3G, kaempferol 3-O-glucoside; Fe²⁺, iron control. Letters above bars indicate significant differences (P<0.05).

Antioxidant Compounds in Dry Beans

are arranged in two orbitals or shells. The innermost shell contains two electrons, and the second and outermost shell contains six electrons. As the body uses oxygen for metabolic reactions, oxygen sometimes gains an extra electron (there are always free electrons floating about a cell seeking to match up with another electron). The extra electron gives oxygen a negative charge because it now has more electrons than protons. This oxygen species with the extra electron is highly unstable and thus, highly reactive. Such molecules or atoms are known as free radicals and characteristically have an unpaired electron or electrons in the outer shell (orbital).³ A free radical always wants to match its unpaired electron(s) by pulling an electron from another atomic species because electrons like to pair up to form stable two-electron bonds.¹⁵ Free radicals are known to be powerful cancer causing agents and can be especially damaging to body tissues and cause degenerative diseases especially in cases where they take an electron from lipid molecules in cell membranes.³ Hydroxyl radicals (another kind of free radical) are especially damaging to cell membranes because they can initiate a process called lipid peroxidation. Lipid peroxidation occurs by radical chain reaction. Once started, the chain reaction spreads rapidly and affects a great number of lipid molecules.¹⁵

Antioxidants Stop Free Radicals

Antioxidants can be viewed as biological scavengers. They seek out and quench free radicals generated by the body's process of metabolism. An antioxidant neutralizes a free radical by donating one of its electrons, and in the case of lipid peroxidation, stopping the chain reaction.^{3,15} When antioxidants lose electrons, they do not become free radicals themselves because they are biologically stable in either the charged

(when they donate an electron) or uncharged form.³

Flavonoid Activity-Structure Relationships

The relative antioxidant activity of flavonoids in bean seed coats (Figure 2) may be explained by their structures and free radical generation in the body. In the body, oxygen can gain an extra electron during the cellular process that reduces it to water. This sequence of events occurs via the electron transport chain in specialized cellular structures called mitochondria. The addition of an extra electron to oxygen generates the free radical called superoxide radical [O₂]. Superoxide radical is also known as reactive oxygen species or ROS. The superoxide radical can gain another electron and react with two hydrogen ions to form hydrogen peroxide, and in the presence of iron or copper atoms, the peroxide can form the highly damaging hydroxyl radical [OH].¹⁶ Therefore, the ability of flavonoids to complex with metals plays a part in their role as antioxidants. As a general rule, the greater the number of hydroxy groups on the flavonoid nucleus, the higher the antioxidant activity.¹⁷

The most important structural feature of flavonoids for antioxidant activity is the B-ring ortho 3',4' dihydroxy orientation (refer to Figure 1).¹⁸⁻²⁰ The two most active flavonoids in the study, delphinidin and petunidin, are anthocyanins. Anthocyanins along with tannins are the flavonoids that give black and purple beans their color. Delphinidin and quercetin have a hydroxy group at the 3',4' positions and petunidin has a 4',5' dihydroxy group. Malvidin, the third anthocyanin found in black beans, has both the 3', and 5' hydroxy groups methylated (OCH₃). The methyl group (CH₃) essentially blocks the hydroxy group (OH), thus, rendering malvidin less effective as an antioxidant than the other flavonoids. Kaempferol, which has only a single B-ring

4'-hydroxyl substitution, has the least antioxidant activity of the flavonoid compounds found in beans.

Conclusions

Research is providing solid evidence that consumption of a variety of phenolic compounds present in natural foods can lower the risk of serious health disorders because of the antioxidant activity of these compounds.^{21,22} Dry beans are an integral part of the diets of people in many countries of the developing world. Statistics show that citizens of developing countries in which legumes are dietary staples have fewer diet related health problems than citizens in developed countries. In view of the positive contribution legume consumption makes to human health and well being, the question may be asked, "Shouldn't diets in the U.S. comprise a larger component of beans than is currently the case?"

The potential "health benefits" from eating beans has been addressed from several points of view; however, the beneficial effects on human health from the wide array of phenolic compounds found in seed coats of colored dry beans largely have been overlooked. The pigments (phenolics) that give bean seed coats their color, are potent antioxidants and may have their greatest benefit on human health by reducing the risk of some types of cancer—colon cancer being one type—by scavenging lipid peroxy radicals. Epidemiological studies have reported that people with high intakes of vegetables and fruits rich in antioxidant compounds have low rates of cancer.²² Also, positive benefits of bean antioxidants on heart health should not be considered trivial. Results of a long-term study of the diet and lifestyle of individuals in the Netherlands, showed that a regular intake of several dietary flavonoids, such as quercetin and kaempferol, reduced the risk of coronary heart disease in elderly men.²²

Although there is no guarantee that eating beans will prevent cancer because of their antioxidant content, there is compelling scientific evidence regarding the free radical quenching effects of bean phenolics to make it worthwhile to include beans as a major component of the diet. A decision to increase the amount of beans in the diet solely because of the ability of flavonoids to neutralize the effects of free radicals takes on added significance when one considers that under normal metabolic conditions, each cell in one's body is exposed to about ten billion molecules of superoxide per day.¹⁵ This amount of daily superoxide exposure extrapolated to an annual basis amounts to about four pounds of free radicals for a person weighing 150 pounds. This is a substantial amount of highly reactive free radicals that can bombard cell membranes (lipids), DNA, and proteins and cause severe cell and tissue damage resulting in debilitating diseases.

To maximize health benefits from eating beans, go for the market classes with the

darker seed coats—the brown and black colored beans. Black beans contain anthocyanins—the most efficacious monomeric antioxidant compounds found in the antioxidant experiment—while brown colored ones contain a goodly amount of tannins, which have potent antioxidant effects.² Black beans are robust in flavor, and combine nicely with many other ingredients in recipes. Indeed, black beans are not only appealing for their culinary qualities, but they also have tremendous potential for conferring health benefits to those who eat them on a regular basis. So! "Turn up the crock pot and bring on the feijoada!"

References

1. Geil, P. B., and Anderson, J. W. (1994) Nutrition and health implications of dry beans—a review. *J. Am. Coll. Nutr.* 13, 549–558
2. Beninger, C. W., and Hosfield, G. L. (2003) Antioxidant activity of extracts, condensed tannin fractions, and pure flavonoids from *Phaseolus vulgaris* L. seed coat color genotypes. *J. Agric. Food Chem.* 51, 7879–7883
3. Whitney, E. N., and Rolfes, S. R. (1999) *Understanding Nutrition*, 8th ed. West/Wadsworth, Belmont, CA, 649 pp
4. Buchanan, B. B., Gruissem, W., and Jones, R. L. (2000) Biochemistry and molecular biology of plants. *Am. Soc. Plant Physiol.*, Rockville, MD, 1367 pp
5. Feenstra, W. J. L. (1960) Biochemical aspects of seedcoat colour inheritance in *Phaseolus vulgaris* L. Med. Landbouwhogeschool Wageningen. 60, 1–53
6. Beninger, C. W., Hosfield, G. L., and Nair, M. G. (1997) Phytochemical methods for the extraction and analysis of seed coat flavonoids from common bean (*Phaseolus vulgaris* L.) 1997 Annu. Rep. Bean Improv. Coop. 40, 17–18
7. Beninger, C. W., Hosfield, G. L., and Nair, M. G. (1998) Flavonol glycosides from the seedcoat of a new Manteca type dry bean (*Phaseolus vulgaris* L.). *J. Agric. Food Chem.* 46, 2906–2910
8. Beninger, C. W., and Hosfield, G. L. (1999) Astragalin (kaempferol 3-O-glucoside) and proanthocyanidins are the main flavonoid compounds of four *Phaseolus vulgaris* L. seed coat color genotypes. Annu. Rep. Bean Improv. Coop. 42, 119–120
9. Beninger, C. W. and Hosfield, G. L. (1999) Flavonol glycosides from Montcalm dark red kidney bean: implications for the genetics of seed coat color in *Phaseolus vulgaris* L. *J. Agric. Food Chem.* 47, 4079–4082
10. Beninger, C. W., Hosfield, G. L., and Bassett, M. J. (1999) Flavonoid composition of three genotypes of dry bean (*Phaseolus vulgaris* L.) differing in seed coat color. *J. Am. Soc. Hortic. Sci.* 124, 514–518
11. Beninger, C. W., Hosfield, G. L., Bassett, M. J., and Owens, S. (1999) Chemical and morphological expression of the B and Asp seedcoat genes in *Phaseolus vulgaris* L. *J. Am. Soc. Hortic. Sci.* 125, 52–58
12. Hagerman, A. E., Riedl, K. M., Jones, G. A., Sovik, K. N., Ritchard, N. T., Hartzfeld, P. W., and Riechel, T. L. (1998) High molecular weight plant phenolics (tannins) as biological antioxidants. *J. Agric. Food Chem.* 46, 1887–1892
13. Ariga, T., Koshiyama, I., and Fukushima, D. (1988) Antioxidative properties of procyanidins B-1 and B-3 from adzuki beans in aqueous systems. *Agric. Biol. Chem.* 52, 2717–2722
14. Ganthavorn, C., and Hughes, J. S. (1997) Inhibition of soybean oil oxidation by extracts of dry bean (*Phaseolus vulgaris* L.). *J. Am. Oil Chem. Soc.* 74, 1025–1030
15. Frei, L. (2003) Reactive oxygen species and antioxidant vitamins. <http://lpi.oregonstate.edu/f-w97/reactive.html>
16. Duthie, G. G. (1993) Lipid peroxidation. *Eur. J. Clin. Nutr.* 47, 759–764
17. Cao, G., Sofic, E., and Prior, R. L. (1997) Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationships. *Free Radical Biochem. Med.* 22, 749–760
18. Dziedzic, S. Z., and Hudson, B. J. F. (1983) Polyhydroxy chalcones and flavanones as antioxidants for edible oils. *Food Chem.* 12, 205–212
19. Dziedzic, S. J., and Hudson, B. J. F. (1984) Phenolic acids and related compounds as antioxidants for edible oils. *Food Chem.* 14, 45–51
20. Letan, A. (1966) The relation of structure to antioxidant activity of quercetin and some of its derivatives. II. Secondary (metal-complexing) activity. *J. Food. Sci.* 31, 395–399
21. Keli, S. O., Hertog, M. G. L., Feskens, E. J. M., and Kromhout, D. (1996) Dietary flavonoids, antioxidant vitamins and incidence of stroke—The Zutphen study. *Arch. Intern. Med.* 156, 637–642
22. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., and Kromhout, D. (1993) Dietary antioxidant flavonoids and risk of coronary heart disease—The Zutphen Elderly study. *Lancet* 342, 1007–1011



1031 South US-27 • St. Johns, Michigan 48879
Phone (989) 224-1361 • Fax (989) 224-6374

www.michiganbean.org