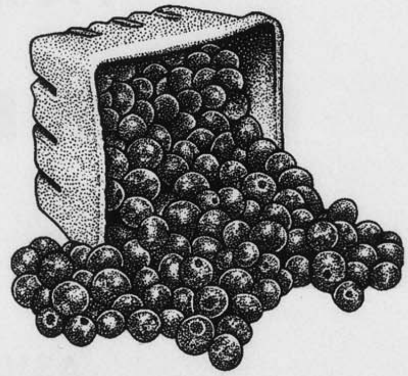
# Health Functionality of Blueberries



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**Additional index words.** anthocyanin, flavonoid, bilberry, medicinal

**Summary.** Blueberries (*Vaccinium* sp.) have a long history of use in native and folk medicine in North America and Europe. Today the European blueberry (bilberry) is used in a variety of pharmaceutical and food supplement products that are recommended for treating blood vessel disorders and ophthalmological conditions. Anthocyanins, the pigments that impart the blue color to blueberries, are considered the active ingredient in bilberry health products, although other related flavonoids are biomedically useful. *Vaccinium* flavonoids are antioxidants and are also recognized for their anticarcinogenic properties and usefulness in treating urinary tract infections. The most immediate, and perhaps greatest, opportunity for a health market for North American blueberries may be in promoting blueberries as a healthy food. As researchers continue to explore the biomedical usefulness of blueberries, the blueberry food industry should strive to retain the healthful phytochemical in their products.

The use of blueberries as a food and medicine has a long history in North America. Food use of wild highbush (*Vaccinium corymbosum* L.) and lowbush (*V. angustifolium* Ait.) by indigenous people is indicated in early records of North American settlers. Early North Americans consumed fresh blueberries and used sun-dried fruit in breads, cakes, and stews. Dried berries were crushed together with fish or meat and fat to make a nutritious, dried meat cake called pemmican (Turner, 1991). Blueberries, as a tea or syrup, were used medicinally as a cough treatment, a diarrhea remedy, and for various female illnesses (Pellerro Society, 1974).

The European blueberry, or bilberry (*V. myrtillus* L.), grows widely in Scandinavia, eastern Europe, and at higher elevations in southern Europe. Earliest records of the medicinal use of the bilberry date from the Middle Ages, and bilberry fruit and leaves have been used in conventional folk medicine in Europe since the 16th century (Morazzoni and Bombardelli, 1996).

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The fruit of bilberries were used for their astringent and antiseptic properties, to treat inflammations and infections, to combat scurvy, and to treat urinary complaints. The leaves of bilberry were also used as an anti-inflammatory and antiseptic and to regulate blood sugar levels in the treatment of diabetes (Morazzoni and Bombardelli, 1996 ). European bilberry is described in many pharmacopoeias, and its medicinal use is the basis of an extensive industry in pharmaceutical and health products in Europe and Asia (Morazzoni and Bombardelli, 1996).

## Nutritional quality

The mineral and vitamin content of lowbush, highbush, rabbiteye, and European blueberries has been reported (Bushway et al., 1983; Eitenmiller et al., 1977; Pellerro Society, 1974; USDA, 1981). These commercial species of blueberries are noted for their ascorbic acid content, which has been reported to range between 7 and 20 mg/100 g fresh weight. Cultivar differences in vitamin and mineral content have been noted for rabbiteye blueberries (Eitenmiller et al., 1977) and are likely to exist among genotypes of other blueberry species. Relatively high Mn levels have been reported in lowbush blueberries (Bushway et al., 1983). Manganese is required for the synthesis of certain metalloenzymes, e.g., superoxide dismutase. The Wild Blueberry Association of North America (WBANA) conducted a nutritional evaluation of lowbush blueberries in 1992, and the North American Blueberry Council (NABC) is completing a revised nutritional analysis of highbush blueberries.

## Usefulness in urinary tract infections

Blueberry and cranberry juice are reported to contain a component that is useful in treating urinary tract infections. *Escherichia coli*, a common causal agent for urinary infections, adheres to the wall of the bladder and urinary tract, allowing increased bacterial colonization. A component found in juices of *Vaccinium* fruit acts as an anti-adhesin, blocking the binding of the bacteria to the urinary tract wall and thereby reducing infection (Ofek et al. 1991).

## Anticarcinogenic components

The fruit of certain *Vaccinium* species have recently been reported to contain components that may have anticarcinogenic properties (Bomser et al., 1996). In this study, *Vaccinium* fruit were fractionated and tested in vitro in a screening test for potential anticarcinogenic compounds. One *Vaccinium* fruit fraction was active in inducing an enzyme that protects against xenobiotic-induced cancer. The unidentified compound was more active in bilberries than lowbush blueberries. Another fraction, containing proanthocyanidins, was active in inhibiting an enzyme that is characteristic of rapidly growing cancerous cells; in this case, the lowbush blueberries were more active than the bilberry. The results suggest that specific components of some *Vaccinium* species may possess anticarcinogenic activity.

## Biological properties of anthocyanins

Phenolic acids, their esters, and flavonoids are the major phenolic components of blueberries. Included among the flavonoids found in blueberries are flavonols, flavans, proanthocyanidins, and anthocyanins. Anthocyanins are pigments responsible for the red, violet, and blue colors of various plant parts, including blueberry fruit. Of sixteen different anthocyanidins found in nature, six are most common in plants. Anthocyanidins always occur in glycosidic linkages with one or more sugar molecules, giving rise to a great number of different anthocyanidin glycosides (anthocyanins). These various glycoside forms and other anthocyanidin substituents, as well as various chemical interactions, give rise to the vast array of colors that characterize these pigments. Compared to other fruit, ripe blueberries have a high level of anthocyanin pigments, which is apparent by their deep coloration.

In addition to their role as pigments, anthocyanins possess other interesting biological characteristics. A great deal of research has focussed on the properties of *Vaccinium* anthocyanins, particularly those in the bilberry. Anthocyanins have been investigated in relation to several biological activities including antioxidant capacity, effect on capillary permeability and fragility, effect on blood platelet aggregation, and effect on collagen.

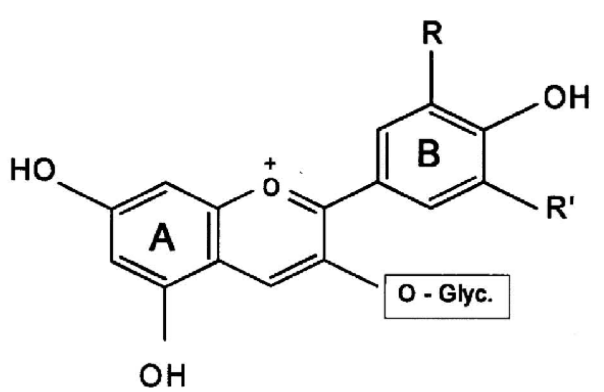
The antioxidant capacity of anthocyanins may be one of their most significant biological properties. Biomedical and epidemiological research suggest that the dietary antioxidants contained in fruit and vegetables may play an important role in preventing disease (Wang et al., 1996). Oxidative damage to lipids, proteins, and nucleic acids has been implicated in the development of cancer, cardiovascular disease, and several other pathological disorders. Anthocyanins and other flavonoids make a substantial contribution to total antioxidant in the diet (Ramararathnam and Osawa, 1996). Flavonoid antioxidants are inherently more stable than the other major fruit antioxidant, Vitamin C (Miller et al., 1995). Flavonoids act as antioxidants by scavenging free radicals, donating electrons to terminate free-radical chain reactions, and chelating pro-oxidant metal ions (Ramararathnam and Osawa, 1996). Antioxidant efficacy of flavonoids is influenced by the number of hydroxyls on the B ring of the molecule (Rajalakshmi and Narasimhan, 1996) so that delphinidin and cyanidin are the more potent of the common anthocyanidins (Morazzoni and Bombardelli, 1996) (Fig. 1).

Bilberry anthocyanins decrease the permeability and fragility of capillaries (Lietti et al., 1976). This property of anthocyanins is common to other flavonoids and was originally characterized by Albert Szent-Gyorgyi, who coined the term Vitamin P for those compounds that reduce capillary permeability (Gábor, 1988). An increase in capillary permeability is one of the first manifestations of the inflammatory process and is a secondary effect of numerous pathological conditions.

Flavonoids have been shown to inhibit blood platelet aggregation (Beretz and Cazenave, 1988), and this effect has been reported for bilberry anthocyanins specifically (Morazzoni and Magistretti, 1990 ). The aggregation of platelets in blood vessels plays a role in thrombosis (blood clotting), and thrombosis is an element in the development of atherosclerosis (Beretz and Cazenave, 1988). Flavonoids affect the adhesion of platelets directly and also through indirect effects on prostaglandin metabolism (Beretz and Cazenave, 1988). The effect of flavonoids on prostaglandin metabolism may be related to the vascular smooth muscle relaxing activity reported for anthocyanins (Morazzoni and Bombardelli, 1996).

**Fig. 1. Structure of anthocyanins.**

Anthocyanidins




| Anthocyanidin | R | R' |
|---|---|---|
| Cyanidin | OH | H |
| Delphinidin | OH | OH |
| Malvidin | $OCH_3$ | $OCH_3$ |
| Pelargonidin | H | H |
| Peonidin | $OCH_3$ | H |
| Petunidin | OH | $OCH_3$ |

Anthocyanins are also reported to have direct and indirect effects on collagen, which is the major protein component of connective tissue (e.g., tendons and cartilage). Anthocyanins cross-link and thereby strengthen the collagen matrix; they have also been reported to inhibit enzymes that break down collagen (Morazzoni and Bombardelli, 1996; Pizzorno and Murray, 1987). The antioxidant property of anthocyanins is reported to serve a protective function in free radical-mediated collagen damage (Monboisse et al., 1984).

The lack of toxic side-effects of anthocyanins is discussed by Timberlake and Henry (1989). The toxicology of bilberry fruit and leaf extracts is discussed in detail by de Smet (1993), and their use is considered safe.

## Clinical applications of anthocyanins

Bilberry anthocyanin extract is used in several clinical applications, mainly in the areas of ophthalmology and vasoprotection. Bilberry extract is reported to improve night-time visual acuity, to aid in adapting more quickly to low light conditions, and to decrease recovery time after exposure to glare (Morazzoni and Bombardelli, 1996 ). Its use is recommended to pilots, truck drivers, and those suffering from poor night vision. Bilberry extract products have become very popular in Japan, where they are used to relieve eye strain and are recommended for those using video display terminals for extended periods. Bilberry fruit anthocyanin extract is reported to prevent glaucoma, and to be effective in the treatment of retinopathies, including diabetic retinopathy (Morazzoni and Bombardelli, 1996; Pizzorno and Murray, 1987). Clinical trials have demonstrated the efficacy of bilberry extract in the treatment of peripheral vascular disease, i.e., reduced blood supply to the lower limbs and in the pre- and postoperative treatment of varicose veins and hemorrhoids (Morazzoni and Bombardelli, 1996). Bilberry extract is also reported to be useful in the treatment of inflammatory conditions of the joints, due to its positive effects on collagen structure and metabolism (Pizzorno and Murray, 1987).

## The bilberry health products industry

An on-line search of a pharmaceutical data base indicated that 184 *Vaccinium* pharmaceutical products have been introduced throughout the world since the beginning of the century (IMS Global Services, 1995). More than 50% of these products were introduced in the last 25 years, and essentially all of them contained the extract of *V. myrtillus*. Bilberry extract was one of five or fewer components in more than 50% of the products, suggesting that it made a substantial contribution to the content of many of the products. Products were specified on the basis of their content of anthocyanosides or active principles. France, Italy, Germany, and Korea have been particularly active in the development of bilberry products. Annual sales of pharmaceutical products containing bilberry extract is in the tens of millions dollars. In addition to this market in prescription and nonprescription products, dietary supplements of bilberry extracts are widely marketed. Although it is difficult to obtain figures on the size of this industry, it appears that all major North American herbal product companies market a bilberry anthocyanin product.

## Technology for anthocyanin extraction for pharmaceutical products

Anthocyanins are highly reactive molecules that can degrade or undergo a variety of reactions that may detriorate their quality in drug products. For example, at a high concentration, anthocyanins will self-associate, forming polymeric products that may not be as effectively metabolized by the body. Industrial-scale procedures for manufacturing anthocyanin extracts for pharmaceuticals are designed to maximize the yield of monomeric anthocyanins, and different processes are available depending on the extract quality required. In one process, a preliminary maceration and aqueous extraction of the peel and flesh is carried out, followed by fermentation of the extract using, for example, *Saccharomyces* sp. to remove free sugars. The extract is then clarified and concentrated, and a second extraction of the remaining solid material is carried out using acidified alcohol. The extracts are brought to dryness by removing solvent under vacuum at a low temperature; this dry material is then crushed, powdered, and packaged. Alternatively, acidified alcoholic extracts may be treated with $SO_2$ to stabilize anthocyanins, followed by nonionic chromatographic separation of anthocyanins from other extraneous components. Solvent is removed and the dried material is prepared based on a standardized anthocyanin content.

The final extract is standardized to contain 25% anthocyanins and the yield of powdered extract from bilberry fruit is 0.2% to 4 %. Two European companies, one in France and one in Italy, are the main producers of bilberry anthocyanin extract used in pharmaceutical and health products. Together they use about 2000 tons of bilberries per year to produce about 30 tons of anthocyanin extract. This extract is used in products made by these companies or by other licensed laboratories.

## Opportunities for North American blueberries

Bilberries have a higher anthocyanin content than the fruit of commercial North American blueberry species. Anthocyanin is present in the peel and flesh of the bilberry, while it is only in the peel of the lowbush, highbush, and rabbiteye blueberries. Table 1 indicates the range of anthocyanin content among bilberries and some North American blueberries. Differences in fruit size and amount of peel per gram fresh weight distinguish the high and lowbush blueberry in terms of their anthocyanin content. Genotypic variability in anthocyanin content within a species is apparent, and, although data in Table 1 is from 1 year only, seasonal differences occur (Kalt and McDonald, 1996). Anthocyanins continue to be formed in the fruit after harvest (Basiouny and Chen, 1988; Kalt and McDonald, 1996). The anthocyanin content of the rabbiteye variety Tifblue (Table 1) was measured after 15 days in storage.

The anthocyanins of bilberries and North American blueberries are generally similar. Five of the six major fruit anthocyanidins are found in blueberries. The major bilberry anthocyanins include delphinidin, cyanidin, petunidin, peonidin, and malvidin, glycosidically linked to either glucose, arabinose, or galactose for a total of 15 (Martinelli et al., 1992). Lowbush blueberries are reported to contain the same 15 anthocyanosides (Francis et al., 1966); however, as chromatographic techniques for anthocyanin separation improve, better resolution and more differentiation of anthocyanin profiles continue to be reported. Gao and Mazza (1994) recently identified acylated anthocyanins in lowbush

**Table 1. Total anthocyanin concentration in selected blueberry samples.**[z]

| Sample | Anthocyanin concn | |
|---|---|---|
| | $mg \cdot g^{-1}$ Fresh wt | $mg \cdot g^{-1}$ Dry wt |
| Bilberry clonal mix | 3.70 | 23.76 |
| Lowbush clonal mix | 1.88 | 13.96 |
| Lowbush Blomidon | 0.954 | 8.37 |
| Lowbush Cumberland | 1.53 | 11.96 |
| Lowbush Fundy | 2.55 | 21.77 |
| Highbush Bluecrop | 0.832 | 6.70 |
| Highbush Coville | 0.998 | 7.01 |
| Highbush Jersey | 1.17 | 8.35 |
| Rabbiteye Tifblue[y] | 2.10 | |

[z]Mean of three samples, calculated as malvidin 3-glucoside.
[y]Reported in Basiouny and Chen, 1988.

blueberries. Highbush and rabbiteye blueberries contain varying amounts of the same 15 anthocyanins as bilberry (Ballington et al., 1987). The particular profile of anthocyanins may not be a critical factor in the biomedical efficacy of a particular fruit extract. Because of the complex mixture of anthocyanins found in bilberries, the biological properties of these molecules are considered to be representative of all anthocyanins (Timberlake and Henry, 1989). Except for a hypoglycaemic component of bilberry leaf extract, where delphinidin 3-glucoside (myrtillin) is considered to be the most active component (Pizzorno and Murray, 1987), no specific properties of any one anthocyanin have been reported.

Compared to the lowbush industry, which is based on wild clones, the highbush and rabbiteye blueberry industries may be better suited to develop new varieties with a high anthocyanin content intended for health-related markets. Factors such as high anthocyanin-forming capacity, early color development, small fruit size, synchronous ripening (Sapers et al., 1986 ), and postharvest anthocyanin formation may be of considerable importance in developing methods to increase fruit anthocyanin content. Other production management factors could also affect fruit anthocyanin content.

The development of North American blueberry pharmaceutical products based on anthocyanin or other fruit components with specific health claims is unlikely at this point due to the extensive and costly process of research, development, and regulatory testing. However the emerging trend in functional foods may represent a more immediate and promising opportunity for the blueberry industry. Functional, or nutraceutical foods, are foods or food ingredients that provide a medical or health benefit beyond the traditional nutrients that they contain. Japan leads the way in recognizing, through a formal licensing and approval system, several categories of food components that have a health benefit. Flavonoids are recognized as a functional food component in Japan (K. Lapsley, personal communication). The U.S. Food and Drug Administration has proposed a process to allow specific health claims for food and, within this process, data is now being gathered for a specific health claim for cranberry juice (K. Lapsley, personal communication). Regardless of progress in regulating functional foods, consumers are becoming more aware, through various information sources, of the significant role of diet in maintaining health and of the potential health benefits of particular foods. The North American blueberry food industry can meet this interest by providing blueberry food products in various forms, with a health-conscious consumer in mind. The blueberry industry may continue to explore the health benefits of its product and possibly benefit from the successful models of the bilberry and cranberry industries.

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