PHENOL COMPOUNDS - QUALITATIVE INDEX IN SMALL FRUITS

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ABSTRACT
Over the past 10 years, researchers have become increasingly interested in polyphenols. The main reason for this interest is the recognition of the antioxidant properties of polyphenols, their great abundance in our diet, and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular and neurodegenerative diseases (51). Furthermore, polyphenols, which constitute the active substances found in many plants, modulate the activity of a wide range of enzymes and cell receptors (1). In this way, in addition to having antioxidant properties, polyphenols have several other specific biological actions that are poorly understood.

Not all polyphenols are absorbed with equal efficiency. Knowledge of the bioavailability and metabolism of the various polyphenols is necessary in order to evaluate their biological activity within target tissues. The types and distribution of polyphenols in foods and the bioavailability of polyphenols are the topics of our knowledge. Several thousand molecules having a polyphenol structure have been identified in higher plants, and several hundred are found in edible plants. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. These compounds may be classified into different groups as a function of the number of phenols that they contain.

This review aims to describe evidences for the effects of polyphenol consumption on health, and to specify which out of the hundreds of existing polyphenols are likely to provide the greatest protection in the context of preventive nutrition. If these objectives are to be attained, it is first essential to determine the nature and distribution of these compounds in fruits. Such knowledge will allow evaluation of polyphenol intake and enable epidemiologic analysis that will in turn provide an understanding of the relation between the intake of these substances and the risk of development of several diseases.

Keywords: small fruits, phenol compounds, qualitative index


Introduction
For many plant products, the polyphenol composition is much less known, knowledge is often limited to one or a few varieties, and data sometimes do not concern the edible parts. Some foods, particularly some exotic types of fruit and some cereals, have not been analyzed so far.

Berry fruits, such as bilberry (Vaccinium myrtillus), blackberry (Rubus fruticosus), blackcurrant (Ribes nigrum), blueberry (Vaccinium corymbosum), chokeberry (Aronia melanocarpa), cranberry (Vaccinium macrocarpon), grape (Vitis vinifera), raspberry (Rubus idaeus) and strawberry (Fragaria x ananassa) are a particularly rich source of antioxidants (13, 25, 27, 28, 30, 40, 50). Those compounds are mainly represented by vitamin C and polyphenols such as anthocyanins, phenolic acids, flavonols and tannins. They are known as natural antioxidants and due to their high concentration and qualitative diversity, berry fruits are increasingly often referred as natural functional foods.

Phenolic compounds are the secondary metabolites of plant provided their normal growth and development. Many researchers reported that the fruits of berry plants which grow in a cold northern climate with a short vegetation season, without fertilizers and pesticides are marked by a higher content of polyphenols than the same varieties which grow in a milder climate. In addition to their protective functions, phenolic compounds /in particular- anthocyanins/ are responsible for the pigmentation of flowers, fruit and leaves (15, 16, 17).

Anthocyanins in berry fruits comprise a large group of water-soluble pigments. In the fruit, they are found mainly in the external layers of the hypodermis (the skin). In the cells, they are present in vacuoles in the form of various sized granules; meanwhile cell walls and flesh tissue practically not contain anthocyanins.

Factors influencing on polyphenol content
Numerous factors other than variety may affect on the polyphenol content in plants. The content of phenolic compounds in berry fruits is determined by many factors too, such as the species, variety, cultivation, region, weather conditions, ripeness, harvesting time, storage time and conditions (3, 11, 19, 21, 31, 32, 37, 45, 47).

Environmental factors have a major effect on polyphenol content. These factors may be climatic (soil type, sun exposure, rainfall) or agronomic (culture in greenhouses or fields, biological culture, hydroponic culture, fruit yield per tree, etc). Exposure to light has a considerable effect on most flavonoids. The degree of ripeness considerably affects the concentrations and proportions of the various polyphenols (34). In general, phenolic acid concentrations decrease during ripening, whereas anthocyanin concentrations increase.

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polyphenols, especially phenolic acids, are directly involved in the response of the plants to different types of stress: they contribute to healing by lignification of damaged areas, they possess antimicrobial properties, and their concentrations may increase after infection (2, 39, 49). Although very few studies directly addressed this issue, the polyphenol content of vegetables produced by organic or sustainable agriculture is certainly higher than that of vegetables grown without stress, such as those grown in conventional or hydroponic conditions.

This was shown recently in strawberries, blackberries and corn (4). With the current state of knowledge, it is extremely difficult to determine for each family of plant products the key variables that are responsible for the variability in the content of each polyphenol and the relative weight of those variables. A huge amount of analysis would be required to obtain this information. For example, determination of the p-coumaric acid content of 500 red wines showed that genetic factors were more important than was exposure to light or climate (9).

Storage may also affect on the content of polyphenols that are easily oxidized. Oxidation reactions result in the formation of more or less polymerized substances, which lead to changes in the quality of foods, particularly in color and organoleptic characteristics. Such changes may be beneficial (as is the case with black tea) or harmful (browning of fruit) to consumer acceptability. Storage of wheat flour results in marked loss of phenolic acids. After 6 months of storage, flours contained the same phenolic acids in qualitative terms, but their concentrations were 70% lower. Cold storage, in contrast, did not affect on the content of polyphenols in apples (7, 51), pears (58) or onions (64). At 25°C, storage of apple juice for 9 months results in a 60% loss of quercetin and a total loss of procyanidins, despite the fact that polyphenols are more stable in fruit juices than is vitamin C (60, 61).

Methods of culinary preparation also have a remarkable effect on the polyphenol content of foods. For example, simple peeling of fruit and vegetables can eliminate a significant portion of polyphenols because these substances are often present in higher concentrations in the outer parts than in the inner parts. Cooking may also have a major effect. Onions and tomatoes lose between 75% and 80% of their initial quercetin content after boiling for 15 min, 65% after cooking in a microwave oven, and 30% after frying (13). Steam cooking of vegetables, which avoids leaching, is preferable.

Industrial food processing also affects on polyphenol content. As with fruit peeling, dehulling of legume seeds and decortication and bolting of cereals can result in a loss of some polyphenols. Then they are transformed into brown pigments that are polymerized to different degrees.

This unwanted process can occur, for example, during the process of making jam or compote from fruit. Production of fruit juice often involves clarification or stabilization steps specifically aimed at removing certain flavonoids responsible for discoloration and haze formation. Manufactured fruit juices thus have low flavonoid content.

Because of the wide range of existing polyphenols and the considerable number of factors that can modify their concentration in foods, no reference food-composition tables (as they exist for other micronutrients such as vitamins) have yet been drawn up. Only partial data for certain polyphenols, such as flavonols and flavones, catechins, and isofoflavones, have been published on the basis of direct food analysis (2, 22, 26) or bibliographic compilations (48, 49). Since March 2003, a database in which the flavonoid contents of 225 selected foods were compiled from 97 bibliographic sources has been available on the US Department of Agriculture website A comprehensive composition table for polyphenols is essential; it should allow daily polyphenol consumption to be calculated from dietary questionnaires (64). Polyphenol intake could then be correlated with the incidence of certain diseases or early markers for these diseases in epidemiologic studies, which would permit investigations of the protective role of these micronutrients.

Polyphenol concentrations in foods may vary according to genetic, environmental, and technologic factors, some of which could be controlled in order to optimize the polyphenol content of foods.

The health effects of polyphenols depend on both their respective intakes and their bioavailability, which can vary greatly. However, values for these variables do not seem to be well correlated with concentrations measured in tissues. Research on polyphenol bioavailability must finally allow us to correlate polyphenol intakes with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma and tissues) and with potential health effects in epidemiologic studies. Knowledge of these correlations must be attained despite the difficulties linked to the high diversity of polyphenols, their different bioavailabilities, and the high interindividual variability observed in some metabolic processes, especially those in which the microflora is involved.

The flavonols are the most diverse flavonoids in the foods. Regarding quantitative point of view, their main representatives are quercetin and kaempferol. Berry extracts of several cultivars of blackberries, black and red currents, blueberries and black and red raspberries showed a remarkably high scavenging activity toward chemically generated superoxide radicals.

**Biological Activity and Medicinal Properties of the Bioactive Compounds of Berry Fruits**

The diversity of bioactive compounds found in berry fruits and processed fruit products is reflected in the broad spectrum of their biological and medicinal properties. Raspberry and chokeberry fruits and its products supplement the treatment of hypertension, atherosclerosis and gastrointestinal tract disorders. The bioactive compounds found in chokeberry strengthen blood vessel walls and improve their elasticity. The juice improves peripheral circulation of the blood and boosts the body’s resistance to infections (23, 24).
The compounds found in this fruits also have a beneficial impact on the circulatory system. Bilberry anthocyanins improve the elasticity and permeability of the capillary vessels of the eyeball, thus improving microcirculation of the blood and vision at dusk and at night. The anthocyanins of bilberry are applied in the production of ophthalmic preparations (49).

Cranberry juice is used in the prevention and treatment of urinary system infections (12, 23, 29, 30), as well as in the treatment of periodontitis (5, 45, 46, 47) and other disorders. Yamanaka et al. (43, 44) reported that cranberry juice can inhibit the colonization of the tooth surface by oral streptococci, and thus slow the development of dental plaque. By using a microplate system, these authors found that the high-molecular weight constituents of cranberry juice inhibited biofilm formation by the tested streptococci.

Lange et al. suggest that cranberry may contribute to the prevention and treatment of periodontitis by reducing the capacity of Porphyromonas gingivalis to colonize periodontal sites (33, 42, 47, 53). Cremin et al. (12) reported that high-molecular weight proanthocyanidins (condensed tannins) from cranberry juice inhibit the adherence of uro-pathogenic fimbriated Escherichia coli and thus offer protection against urinary tract infections. Furthermore, a high-molecular weight cranberry fraction was also reported to inhibit the sialic acid-specific adhesion of Helicobacter pylori to human gastric mucosa, a critical step for gastric ulcer development (5).

In recognition of their biological activity, phenolic compounds have been long used as natural remedies in the treatment of various diseases (circulatory, respiratory, digestive and urinary system ailments). They are applied in the production of various pharmaceutical products due to their ability to seal capillary vessels and improve circulation. Despite widespread research and the documented wide range of biological activity of those compounds, the mechanism responsible for their beneficial effect on the human body has not been sufficiently investigated (14, 15, 18, 20, 22, 54, 55).

In addition to provitamin activity and antioxidant properties, carotenoids, including β-carotene, have several other functions in the body at the molecular level where they act as immunomodulators, inhibit mutagenesis and prevent malignant transformations (56, 57). In addition to its antioxidant properties, vitamin C participates in other important biochemical transformations such as the synthesis of collagen, neurotransmitters and hormones. The Vitamin C is a detoxicant and it neutralizes various mutagenic and cancerogenic compounds which are formed in the alimentary system or which enter the digestive tract with food. Thanks to those properties as well as its antioxidant activity, vitamin C is regarded as an effective remedy in inhibiting the development of cancer (1, 6, 10).

Wild small berry fruits bio-active compounds
The potential capacity of bio-active compounds in wild small fruits is in natural populations generally located in the mountain regions. The development of research approaches for evaluation, preservation and utilization of this national riches will be one main priority for all the countries. For example, in the semi-mountain and mountain regions of Bulgaria with altitude of 1200-1500 meters there are many natural habitats of blackberries /red and black/ as well as strawberries and raspberries. Fruits are usually collected from local people and consumed fresh or transferred for processing /for juices and sweets/. The interest in these fruits is only commercial. So far, there are not systematic study for determination of the diversity in polyphenolic profile and its content in the wild small berries fruits related to their genetic differences and exogenous factors such as climatic and soil conditions, method of processing and storage and their effects on human health. According to recent international publications, the scientific interest in wild small fruit crops was increased. Development of a large number of analytical technologies, defines small fruits as a very rich source of biologically active substances such as elagitanins, phenols, flavonoids, lignins as well as complex phenol polymers and organic acids possessed important biological properties (33, 35, 36) There are carried out numerous in vitro studies, which demonstrate that polifenols in small fruit species are powerful food antioxidants (24). It is estimated that these substances are contained in approximately the same amount in fruits and leaves, and have expressed strong medical qualities (preventive, antibacterial and antiviral) (6, 14). Compared with most fruit crops small friuis are rich in antocyanins, which is due to their red, violet, magenta or blue color. Antocyanins in small fruits cultures and their role in terms of cardiovascular disease are investigated in a project financed by the EU (qlk1-1999-00124). Furthermore, rich in antocyanins extracts from small fruits are available in the market as the form of food additives (41, 63). Antioxidant activity depends largely on the type of small fruit crops, as well as the wild species usually have a higher total antioxidant potential than cultivated ones (62). Recently, there were reported significant variations in the content of antocyanins in different populations of wild small berries in Europe (8, 32). Antioxidant preservation of activity in food and drink from wild species depends largely on the terms of processing and storage. Recent reports of the content of natural antioxidants in dark wild species are impressive and significant compared to their content in vegetables and fruit. For example the content in raspberries is 630 mg/100 g, blackberries -250 mg/100 g (49, 56, 59).

Advances in developed analytical methods is an essential prerequisite for the development of “metabolomics” - an information technology for expanding knowledge of the molecular organization in multi-cellular organisms. Metabolomics is a exclusive opportunity to examine changes in phenotypes, resulting impact on the environment, biotic and abiotic stress (14, 62). The most significant role of metabolomics is filling in the missing information in the relationship phenotype - genotype. Basic knowledge accumulated in the metabolomics leaded to extending the methodological basis for analysis of secondary metabolites and development of high analytical methods / MS / MS MS-
Q-TOF / with achieve precision in determining the molecular masses of chemical compounds. In recent years there were developed many research projects aimed at studying the bioactive compounds in small fruit and their characterization in favor of human health (34, 35, 36, 38, 52).

Currently there is limited understanding of the variation of these components, environmental factors affecting their synthesis and the ability to study their genetic basis, their knowledge of biosynthesis and the use of these components as genetic markers for quality fruit. Existence in Bulgaria of extremely valuable resources of small wild fruits requires efforts for effective management. The characterization of the genetic resources of wild small fruits species / blueberry-black and red, raspberry and strawberry / in different ecological regions on the basis of a comprehensive evaluation, including multidisciplinary approaches - evaluation of natural habitats, biotechnological and phytopatological assessment, metabolic profile, DNA analysis, microbiological analyses in order to determine the effects of extracts from these fruits of human pathogens and to develop appropriate technology for processing of fruits aimed to preserve their metabolic profile.

Conclusions

Polyphenol concentrations in foods may vary according to numerous genetic, environmental, and technologic factors, some of which may be controlled to optimize the polyphenol content of foods. The main tasks ahead are identifying the plant varieties that are the richest in the polyphenols of interest, improving growing methods, and limiting losses during the course of industrial processing and domestic cooking. The health effects of polyphenols depend on both their respective intakes and their bioavailability, which can vary greatly. Research on polyphenol bioavailability must finally allow us to correlate polyphenol intakes with one or several accurate measures of bioavailability (such as concentrations of key bioactive metabolites in plasma and tissues) and with potential health effects in epidemiologic studies.

REFERENCES