HANDBOOK OF FOOD BIOENGINEERING
VOLUME 11

DIET, MICROBIOME AND HEALTH



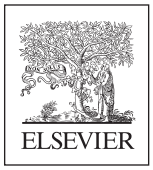
Edited by
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Diet, Microbiome and Health

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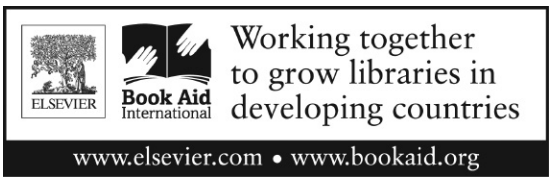
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CHAPTER 7

Flavonoids in Foods and Their Role in Healthy Nutrition

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1 Introduction

Food is a source of essential chemical compounds and provides energy and a broad spectrum of biologically active compounds for living organisms. It plays a vital role for the human body throughout its life cycle, ensuring growth and development and sustaining its physical and mental activity. Therefore, knowledge of food composition is one of the most important tasks of food and nutrition sciences.

The interest in flavanoids' content in foods started to increase at the beginning of the 1990s after the publications of Steinmetz and Potter (1991), which reported that a diet rich in fruits and vegetables decreases the risk of getting a number of chronic diseases, in particular, cancer. This research induced a series of studies on various compounds in fruits and vegetables, which could contribute to the aforementioned beneficial health effects. However, the results did not show a correlation between the known macro- and micronutrients and the attention of the researchers focused mainly on the biological value of other nonnutritive food compounds, part of which is the family of flavonoids. Flavonoids are representatives of the large class of phenolic compounds, which are known as powerful antioxidants, and initially the interest in those compounds was mainly due to their pigment properties in plants (von Elbe and Schwartz, 1996). The interest in flavonoids begin to raise in 1993, triggered by the publishing of the first epidemiological survey (Zutphen Elderly Study) that revealed a reverse relationship between the high intake of flavonoids (flavonols and flavones) and risk of cardiovascular disease (CVD) (Hertog et al., 1994). This survey was supported by numerous studies outlining the protective role of flavonoids concerning CVD (Cassidy et al., 2012, 2013; Rautiainen et al., 2012), cancer (Garcia-Closar et al., 1999a,b; Knekt et al., 1997; Kozics et al., 2011) and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (Gao et al., 2012).

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The aim of the chapter is to review the role of flavonoids in healthy nutrition, and to present our own experimental data for flavonoid content (catechins, flavonols, and flavones) in selected Bulgarian fruits and vegetables, as well as results for their ORAC antioxidant activity.

2 Chemistry and Classification of Flavonoids

Flavonoids (from the Latin word *Flavus*—yellow or blonde) are the biggest and most abundant class of phenolic compounds in nature (von Elbe and Schwartz, 1996). Polyphenols are compounds with more than one hydroxyl group bound to one or more benzene rings. Natural polyphenols share a diverse structure, starting from single molecules to highly polymerized structures, such as tannins (Andres-Lacueva et al., 2010). Flavonoids are important plant pigments and the study of their chemical structure and properties started half a century ago by von Kostanecki and A.G. Perkin (Katyal, 1968).

A common structural element in molecules of flavonoids is the diphenilpropane skeleton C_6—C_3—C_6, where two aromatic rings (the phenolic radicals A and B) are connected via a C-3 carbon aliphatic chain Fig. 7.1 (Pinheiro and Justino, 2012).

Flavonoid molecules contain a heterocyclic, oxygen-containing ring, which is condensed with the phenyl radical A in 2-phenylbenzo-pyranes (chromone) or benzodihydropyran (chromane). Therefore, the classification of the flavonoids depends on two major factors—the place of the second phenyl radical bond to the heterocyclic skeleton and the oxidation state of the heterocyclic ring (Fig. 7.2).

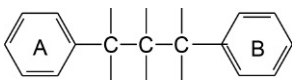


Figure 7.1: Diphenilpropane Skeleton.

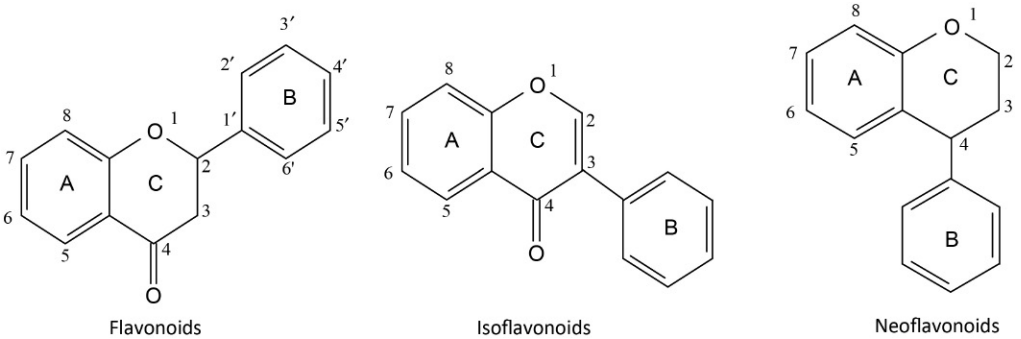


Figure 7.2: Basic Structure of Flavonoids.

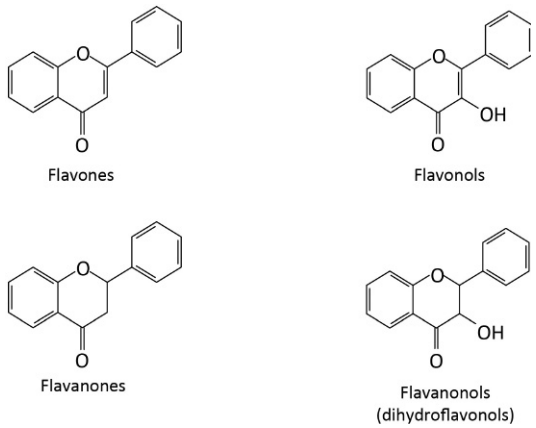


Figure 7.3: Derivatives of 2-Phenylbenzo-Pyranes.

Usually, the phenolic radical B in the flavonoids' structure is bound to a C-2 carbon atom of the chromane or chromone. The bond at the C-3 position (3-phenylbenzo-pyranes) forms a group of isoflavonoids and at the C-4 position, a group of neoflavonoids (Pal and Verma, 2013).

There are four major groups of flavonoids, classified in accordance with the oxidation state of the oxygen-containing ring: derivatives of 2-phenylbenzo-pyranes (flavones, flavonols, and flavanones) and derivatives of benzodihydropyran (flavan-3-ols, flavan-3, 4-diols, anthocyanidins, and desoxyantocyanidins), which are visualized in Figs. 7.3 and 7.4.

The classes of flavonoids differ based on the number and the position of substitutions in aromatic rings A and B. The main functional groups in the structure of flavonoids are the

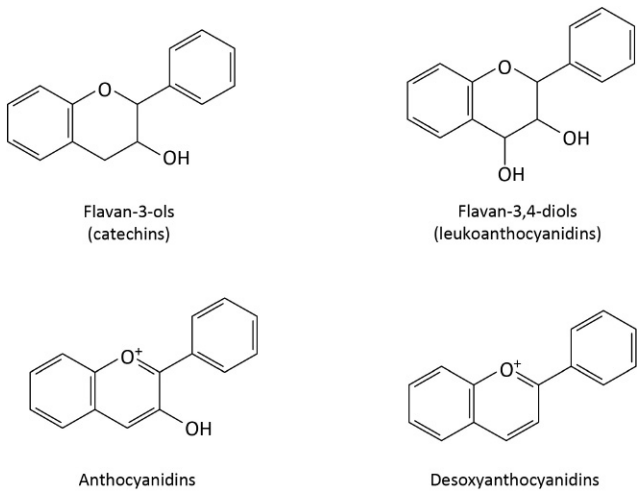


Figure 7.4: Derivatives of Benzodihydropyran.

hydroxyl groups (OH^-) that could be methylated or glycosylated. The hydroxyl substituents in aromatic ring A are mostly bonded at the 5th and 7th atom and are generally not methylated. The hydroxyl groups in the B phenyl radical are mostly bound at C-4′ (phenolic structure), at C-3′, C-4′ (catechol structure) or at C-3′, C-4′, C-5′ (pyrogallol structure) (Rice-Evans et al., 1996; Zhang et al., 2013). The latter bond is relatively rare in the nature because of the relative instability of chemical compounds containing three vicinal OH^-groups.

Flavonoids in plants are rarely in free states, but glycosylated with various carbohydrate moieties. Only leucoanthocyanins and flavan-3-ols are free aglycones and thus can polymerize to various extents to complex structures called proanthocyanidins or condensed tannins (Kassim et al., 2011). The monomeric flavan-3-ols are also called catechins. The great diversity of the glycosides of flavonoids is determined by the various carbohydrate moieties that are building them. The glycoside moieties could be monosaccharides and their derivatives (glucose, galactose, glucuronic acid, etc.), disaccharides (rutinose, neohesperidose, etc.) or trisaccharides, although the latter are extremely rare (Merken and Beecher, 2000). Flavonoids form two main types of glycosides: O-glycosides and C-glycosides. In O-glycosides, the carbohydrate moieties are bonded to OH^-groups of flavonoid molecules through their β-glycoside group, thus forming flavonoid-β-glycosides. Usually, the carbohydrate moieties in the O-glycosides are connected to the phenolic groups at C-3 and C-7, as well as at the C-3′ and C-4′ atoms. In C-glycosides, the carbohydrate components are connected to the flavonoid molecule by a C—C bond, mostly at C-6 or C-8 positions (Veitch and Grayer, 2011). The solubility of flavonoids varies significantly, depending on the presence and properties of the carbohydrate moieties that are bonded to the main phenolic ring. The glycosides of flavonoids are partially or well soluble in water, bases, and acids, while aglycones are more lypophillic and readily soluble in organic solvents, such as methanol, ethanol, ethyl acetate, chloroform, and ether (Munhoz et al., 2014).

Flavonoid synthesis in plants is induced by both high- and low-energy light radiations; therefore, they can be detected mostly in the leaves and bark of plants and in the skin of fruits and vegetables. Flavonoids are synthesised in all plant organs and tissues, usually as glycosides and localized in the vacuoles of plant cells. The soluble polyphenolic compounds are mostly accumulated in the external tissues of fruits and vegetables (epidermal and subepidermal layer) and in a lesser extent in the internal tissues (mesocarb and pulp) (Petrussa et al., 2013).

3 Antioxidant Activity of Flavonoids

3.1 Oxidants and Antioxidants in the Body

Life on earth is impossible without oxygen, but in high concentrations, it could be toxic to aerobes. Most of the negative effects of oxygen could be attributed to the action of oxygen radicals, which include superoxide ($O_2^{\bullet -}$), hydroxyl ($HO^{\cdot}$), peroxyl ($ROO^{\cdot}$), hydroperoxyl

($HOO^{\cdot}$), and alkoxyl ($RO^{\cdot}$) radicals (Gilbert, 1981). Together with the nonradical singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and ozone (O_3) constitute the group of reactive oxygen species (ROS). ROS, together with the nitrogen, are reactive species (RNS). Peroxynitrite ($ONOO^-$), nitric oxide (NO), peroxynitrate, and so on, are constantly produced in our bodies via numerous physiological reactions and processes (Denev et al., 2012). There are at least six major reactive species that cause oxidative damage in the body. These species are peroxyl radicals, hydroxyl radicals, superoxide anion, hydrogen peroxide, singlet oxygen, and peroxynitrite (Halliwell and Gutteridge, 2007; Kirsch et al., 2003; Rifkind, 2004). Living organisms created a complex biological defense system composed of enzymatic and non-enzymatic antioxidants. The term antioxidant is defined as any substance that in low concentrations, compared to those of an oxidizable substrates, significantly delays or prevents oxidation of that substrate (Halliwell and Gutteridge, 2007). By the mechanisms of their actions, antioxidants are divided into preventive and chain-breaking. Preventive antioxidants act as the first line of defense by suppressing the formation of ROS/RNS. The scavenging antioxidants remove reactive species rapidly before they attack biologically essential molecules. For example, superoxide dismutase converts superoxide radicals to oxygen and hydrogen peroxide, whereas catalase converts hydrogen peroxide to water and oxygen. In contrast, no enzymatic action is known to scavenge peroxyl radicals, hydroxyl radicals, singlet oxygen, or peroxynitrite (Huang et al., 2005). Therefore, to counteract their harmful effects, the body relies on a variety of nonenzymatic antioxidants, such as vitamins C and E, and phytochemicals that scavenge reactive species. Usually, there is balance between the antioxidants and the prooxidants in the body, but several factors, such as nutrition, stress, polluted atmosphere, radiation, smoking, and so on, disrupt the oxidative balance leading to a state called oxidative stress. In cases of oxidative stress, it is necessary to administer exogenous antioxidants through diet. The richest sources of antioxidants are herbs, nuts, fruits, and vegetables. Phenolic substances, carotenoids, and antioxidant vitamins C and E are the biggest contributors to the antioxidant properties of plant foods. In the last few decades, phenolic compounds, namely flavonoids, gained a lot of attention and were subject to thorough research because of their antioxidant properties and beneficial effects beyond their vitamin actions. In the section following, the mechanisms of antioxidant actions of flavonoids are presented.

3.2 Mechanisms of the Antioxidant Activity of Flavonoids

Flavonoids could act either as chain-breaking or preventive antioxidants and exert their antioxidant activity through different mechanisms: scavenging species that initiate peroxidation, chelating metal ions—thus making them unable to generate reactive species—decomposing lipid peroxides, quenching $O_2^{\bullet -}$, breaking the autooxidation chain reactions, and/or reducing localized oxygen concentrations (Kumar and Pandey, 2013; van Acker et al., 1996).

3.2.1 The flavonoids—scavengers of free radicals

It has long been known that flavonoids act like scavengers of free radicals (Lima et al., 2014; Meng et al., 2012; Rice-Evans et al., 1996). Their antiradical activity in water and lipid media has been subjected to thorough research (Bilto et al., 2016; Procházková et al., 2011; Rice-Evans et al., 1996). Flavonoid activity as inhibitors of chain radical peroxidation is performed in two stages (Amić et al., 2013; van Acker et al., 1996). Initially, one of the hydroxyl groups of the molecule (usually the 4′-OH group) is dissociated homolythic or heterolytic, thus leaving a flavonoid radical (aroxyl radical).

Homolytic dissociation of the OH^- group

$$\text{Flavo-OH} \rightarrow \text{Flavo-O}^{\bullet} + {}^{\bullet}\text{H}$$

Heterolytic dissociation of the OH^- group

$$\text{Flavo-OH} \rightarrow \text{Flavo-O}^{\bullet} + e^- + H^+$$

The resulting flavonoid radical is stabilized through resonance conjugation of the unpaired electron with the π-electron system of the benzene ring. The stability of the flavonoid radicals is very important for their antioxidant activity, since the generated radical must have high stability and low reactivity to prevent the initiation of new free radicals in the system. The resulting stable flavonoid radical intercepts the chain reaction, giving various molecular products as a result. This is the so-called stage of interruption of chain radical peroxydation (Rice-Evans et al., 1996; van Acker et al., 1996).

$$\text{R}^{\bullet} + \text{Flavo-O}^{\bullet} \rightarrow \textit{stable nonradical products}$$

The antioxidant activity of flavonoids depends on two main factors: (1) the capacity of the hydroxyl groups to bind to the aromatic ring and to yield hydrogen atoms, which then binds the free radicals and (2) the fact that the system can sustain an unpaired electron through delocalization of the π-electron system.

3.2.2 The flavonoids—inhibitors of singlet oxygen

The oxygen molecule is in triplet state ($^3O_2$) and has two unpaired electrons (one for each of the constituting oxygen atoms), which have parallel spins (Thomas, 1994). Therefore, the oxygen molecule is a by-radical. Nevertheless, the oxygen molecule has low reactivity in biological systems, predominantly containing molecules with dominating covalent bonds (Thomas, 1994). The oxygen molecule can be activated in biological systems through monovalent reduction, which yields, in succession, $O_2^{\bullet -}$, H_2O_2, $HO^{\bullet}$, and $^1O_2$ (Tournaire et al., 1993). The triplet oxygen forms the singlet oxygen ($^1O_2$) in some photosensibilization processes (Huvaere and Skibsted, 2015), and unlike the triplet, the singlet has greater oxidation potential and readily engages in reactions with biomolecules. Flavonoids can

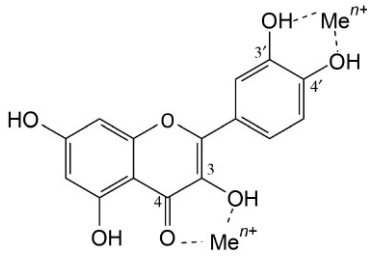


Figure 7.5: Building of Chelate Complexes of 3′,4′-Dixydroxy and 3-Hydroxi, 4-Oxo Groups of Flavonoids With Transitional Metal Ions (Me^{n+}).

inactivate singlet oxygen through its physical transformation to the more stable triplet form or through direct interception of its molecule (scavenging effect) (Kumar and Pandey, 2013; Saewan and Jimtaisong, 2013).

3.2.3 The flavonoids—chelating agents of metal ions

It is well known that ions of transition metals (Fe^{2+}/Fe^{3+}, Cu^{+}/Cu^{2+}) catalyze the formation of free radicals, such as hydroxyl or hydroperoxyl radicals (Mirzaei and Khatami, 2013; Terao and Piskula, 1998). Therefore, binding these ions in stable chelate complexes decreases their role in the initiation of free radical-forming processes. Thus, chelating agents are acting as preventive antioxidants (Terao and Piskula, 1998). Stable chelate complexes of flavonoids and transition metals are mostly formed in the catechol group (3′-OH, 4′-OH) at the B-ring and with the 3-OH and 4-oxo group of the flavonoid molecule (Fig. 7.5).

4 Bioavailability of Flavonoids

The determination of the content and activity of flavonoids in vegetable foods does not provide sufficient characteristics for assessment of their bioefficacy for human and animal health. This deficit is due to the numerous modifications that flavonoids undergo along the gastrointestinal tract, caused by mechanical, chemical, and microbiological impacts. The role of microbiota in the metabolism and assimilation of polyphenolic compounds is the focus of comprehensive studies (Rajilic-Stojanovic et al., 2013; Sekirov et al., 2010). *Bioaccessibility* means the amount of flavonoid released from the food matrix into the gastrointestinal tract, submitted for absorption, and *bioavailability* is the absorbed fraction engaged in the metabolic processes of the organism (Carbonell-Capella et al., 2014; Duda-Chodak et al., 2015). In addition to those terms, it is necessary to also list *bioconversion*, associated with metabolism, and *bioefficacy*, showing the final effect on the organism. The sequence and interrelation of the particular processes depend mainly on gastrointestinal conditions with a focus on the role of various microorganisms residing in certain parts of this route (digestion in the mouth, in the stomach, in the small intestines, in the colon, liver metabolism, transport to target tissues and organs, and excretion).

Many authors have focused their research on the factors that cause changes in the bioaccessibility and bioavailability of bioactive food ingredients. A unified approach to generalized analysis and assessment of this diverse effect has been proposed and is presented by the abbreviation SLAMENGHI. Each letter has its own meaning as follows: *S*pecies of flavonoids, molecular *L*inking, *A*mount of flavonoid, food *M*atrix, *E*ffect modifiers, *N*utrient status, *G*enetic factors, *H*ost-related factors, and mathematical *I*nteractions (van Het Hof et al., 2000).

The assessment of each of the factor listed before contributes to bringing predictable bioefficacy closer to the reality of the studied flavonoid and enables a more correct comparative assessment between the efficacies of the various flavonoid representatives. This system is successfully applicable in the assessment of different nutritive and nonnutritive bioactive compounds in foods.

A number of recent studies are focused on searching for, and the development of, new technologies for the preservation of the native power of flavonoids through implementation of effective encapsulation techniques (Vieira da Silva et al., 2016), implementation of nanoparticles (Akbari and Wu, 2016; Ban et al., 2015; Oehlke et al., 2014), and nanoemulsions (Salvia-Trujillo et al., 2016). The major reason for this trend is the aim to overcome the low bioavailability of many flavonoids.

The final assessment of flavonoids' bioefficacy for human health should consider the following characteristics: composition and content in the dietary intake, bioactivity, bioaccessibility, and bioavailability. At low levels of bioaccessibility and bioavailability it is recommended to engage modern nanotechnologies aiming at maximum preservation of their native bioactivity.

5 Physiological Role and Pharmacological Activities of Flavonoids

In 1936, Rusznyak and Szent-Gyorgi (Nobel Prize winners for the discovery of vitamin C) observed for the first time that scurvy could be treated better with lemons and oranges, rather than with pure ascorbic acid (Rusznyak and Szent-Gyorgy, 1936). They suggested that there was another substance in citrus fruits that supports the normal permeability of blood vessels. This substance was called vitamin P (derived from permeability) or citrine. The chemical analysis of citrine proved that it is composed of two glycosides of the flavonones hesperidin and eriodictyol. At a later stage, similar P-vitamin properties were observed for many flavonoids; for example, they increase the stability of capillaries and their elasticity, and decrease their brittleness and permeability. In the 1950s, flavonoids were excluded from the vitamin classification, because they aren't vitamins, but instead various substances with different structures. Moreover, unlike vitamins, living organisms do not show signs of deficiency for flavonoids if they are excluded from the diet.

There is a vast variety of literary data on the biological activities of flavonoids. In addition to their antioxidant properties, their vasodilatatory (Perez et al., 2014), anticancer (Guo et al., 2013; Ravishankar et al., 2013), antiinflammatory (González et al., 2011; Munhoz et al., 2014), antimicrobial (Kaur and Mondal, 2014; Pistelli and Giorgi, 2012), immune-stimulating (Tao et al., 2014), antiallergenic (Tanaka and Takahashi, 2013), and antiviral activities (Kang et al., 2012) were described. Flavonoids can specifically influence the activity of different enzyme systems, such as cytochrome P450, phospholipase A2, lipoxygenase, etc. (Hodek et al., 2002) and numerous in vitro studies have proven that flavonoids can inhibit and sometimes induce a variety of different enzyme systems. Some of these enzymes are involved in key biochemical pathways regulating cell division and proliferation and thrombocytes aggregation, as well as the processes of detoxification, inflammation, and immune response (Middleton et al., 2000).

Observational and interventional studies have investigated the effect of flavonoids on cardiovascular risk factors, including blood pressure, serum lipids, obesity, diabetes mellitus, and some age-related degenerative disease, such as Alzheimer's disease.

5.1 Flavonoids and Cardiovascular Diseases

Epidemiological studies can be a useful tool for establishing the health effects of polyphenols and in particular flavonoids. A large amount of epidemiological cohort studies examines the relationship between the dietary intake of flavonoids and the risk of cardiovascular disease and reveals results that are unquestionable. A strong inverse relationship between flavonoid intake and CVD was established for the first time in Zutphen, the Netherlands, in 1993 (Hertog et al., 1993). The results from this study were confirmed by the same research team in an international cohort study conducted in seven different countries, showing that the amount of flavonols and flavones in the diet correlates inversely with mortality from CVD (Hertog et al., 1995). A similar correlation was established between the dietary intake of monomer catechins and mortality from CVD in postmenopausal women in Iowa, USA. The prospective study of 34,489 postmenopausal women generated several publications after 10, 13, and 16-year follow-ups (Arts et al., 2001; Mink et al., 2007; Yochum et al., 1999). These studies provided an interesting perspective on that which foods could affect epidemiological results. An inverse relationship between mortality from cardiovascular diseases and the intake of catechin and epicatechin from tea, apples, pears, and chocolate was observed. Individual foods rich in flavonoids that are associated with a significant reduction of mortality from ischemic heart disease and stroke include wheat fiber (associated with stroke), grapefruit (associated with coronary heart diseases), strawberries, and chocolate (associated with cardiovascular disease) (Mink et al., 2007). Another survey carried out in Germany with a 8-year follow-up period of a cohort of 19,357 middle aged men and women reported an inverse relationship between consumption of chocolate and the risk of cardiovascular disease (myocardial arrest and stroke) (Buijsse et al., 2010). The authors observed 106 cases of myocardial infarction and stroke in the quartile characterized with the lowest consumption of

chocolate (1.7 g/day), while in the quartile with the highest intake of chocolate (7.5 g/day) 61 cases were observed (mean relative risk of 0.6 was calculated). The group with high intake of chocolate had significantly lowered systolic and diastolic blood pressure (1 mmHg) compared to the group with low consumption.

A recent study in Sweden (Stockholm Heart Epidemiology Program) investigated the effect of chocolate intake in a group of 1,169 patients without established diabetes, after acute myocardial arrest, with a median duration of follow-up of 8 years (Rautiainen et al., 2012). The high intake of chocolate (50 g more than twice a week) was associated with a reduction of mortality from coronary artery disease. However, possible errors in such surveys could affect the reported results. For example, individuals with unhealthy habits could report a lower intake of chocolate because of guilt. In some cases, nutritional intervention studies based on assumptions generated by epidemiological studies were disappointing. A report by Sesso et al. (2000) in the Physicians' Health Study II states that supplementation with vitamins E and C does not lead to the reduction of the number of CVD diagnoses.

Summarizing the available epidemiological studies, it is evident that one study indicates direct correlation between the intake of tea flavonoids and CVD (Wales relative risk 1.6) (Hertog et al., 1997). Nowadays, it is thought that the reason for these results is a study bias, since in the 1990s, in Wales, the high tea consumption was associated with high smoking level, as well. There are two studies reporting similar correlations (CVD relative risk 1.08, USA, and stroke relative risk 1.18, USA), but the results are within statistical error (Rimm et al., 1996; Yochum et al., 1999).

In all other epidemiological studies, there is an inverse relationship between the intake of flavonoids and CVD. This correlation varies from very strong: 0.32 Zupten, Netherlands (Hertog et al., 1993)—to weak: 0.81 USA (Cassidy et al., 2012)—or almost insignificant: 0.90 (Sweden, USA) (Rautiainen et al., 2012).

Data obtained so far suggests beneficial health effects of flavonoids in relation to CVD. The majority of studies only cover the classes of flavones and flavonols without taking into account other classes of flavonoids and polyphenols. The results regarding stroke are the most controversial, reporting relative risks from 0.27 (Keli et al., 1996) to 1.18 (Yochum et al., 1999). This indicates the need for both new research and development of accurate databases for the content of flavonoids in foods. These databases must be based on actual quantitative analysis of flavonoid composition of foods and food products by applying advanced analytical methods and very strict selection of samples to achieve representative results.

5.2 Flavonoids and Cancer

The anticancer effect of flavonoids has been discussed in numerous scientific publications (Batra and Sharma, 2013; Chahar et al., 2011; Kahali et al., 2014; Vijayalakshmi et al., 2013).

Flavonoids influence several important biological functions, which may explain the observed inverse associations of their intake and cancer risk. Several mechanisms have been proposed for the effect of flavonoids in cancer initiation, promotion, and development: down regulation of mutant p53 protein, cell cycle arrest, tyrosine kinase inhibition, inhibition of heat shock proteins, estrogen receptor binding capacity, and inhibition of the expression of the Ras protein (Kumar and Pandey, 2013).

In recent years, many epidemiological studies aiming to prove the preventive effect of flavonoids against certain types of cancer have been performed. Some studies revealed that flavonoid intake is associated with about a 50% reduction of risk of lung cancer (Knekt et al., 2002), while other did not find correlation between dietary intake of flavonoids and mortality from common cancers—relative risk 1.2 (0.7–2.2) (Hertog et al., 1994).

The literature also provides some case-control epidemiological studies, which examines the effects of dietary intake of flavonoids on cancer. A protective effect of flavonols, flavones, and citrus flavonoids against the development of gastric cancer in Spain (Garcia-Closar et al., 1999a,b) and lung cancer in Hawaii (Le Marchand et al., 2000) was observed, while in two case-control studies conducted in Spain, no relationship between flavonols and flavones intake and lung cancer (Garcia-Closar et al., 1998) or blood cancer (Garcia-Closar et al., 1999a,b) was found.

The recent meta-analysis of 12 studies, involving 9513 cases and 181,906 controls, 6 of which were prospective cohort studies, and 6 case-control studies were done to investigate the relationship between breast cancer and flavonoids intake. The results showed that the total relative risk of breast cancer significantly decreased in women with a high intake of flavonols (RR = 0.88, 95% CI 0.80–0.98) and flavones (RR = 0.83, 95% CI: 0.76–0.91) compared with those with low intake of flavonols and flavones. However, no significant association of flavan-3-ols (RR = 0.93, 95% CI: 0.84–1.02), flavanones (RR = 0.95, 95% CI: 0.88–1.03), anthocyanins (RR = 0.97, 95% CI: 0.87–1.08), or total flavonoids (RR = 0.98, 95% CI: 0.86–1.12) intake with breast cancer risk was observed. Furthermore, the relative risk found in three case-control studies stratified by menopausal status suggested that the intake of flavonols, flavones, or flavan-3-ols is associated with a significant reduced risk of breast cancer in post-menopausal women, while not in premenopausal women. In this study, the intake of flavonols and flavones, but not other flavonoid subclasses or total flavonoids, was associated with a decreased risk of breast cancer, especially among postmenopausal women (Hui et al., 2013). Epidemiological studies suggest that dietary intake of flavonoids may reduce the risk of certain cancers—breast, colon, lung, prostate, and pancreas. However, some studies have reported inconclusive or even harmful associations. A major challenge in the interpretation of epidemiological studies is that most of the data originates from case-control studies and retrospective acquisition of flavonoid intake (Romagnolo and Selmin, 2012).

5.3 Flavonoids and Obesity

According to World Health Organization (WHO), overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health. Furthermore, obesity is associated with the occurrence of low-level chronic inflammation, demonstrating a close link between metabolism and immunity (Al-Goblan et al., 2014; Hossain et al., 2016). Some recent WHO global estimates show that in 2014, more than 1.9 billion adults worldwide were overweight and among them, over 600 million were obese. Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2014 and 39% of adults (38% of men and 40% of women) were overweight. The worldwide prevalence of obesity more than doubled between 1980 and 2014. In 2013, 42 million children under the age of 5 were overweight or obese (WHO, 2015).

Overweight and obesity are linked to more deaths worldwide than underweight, since obesity causes the development of metabolic disorders as diabetes mellitus, hypertension, cardiovascular disease, and inflammation-related pathologies. The early studies on flavonoids and their role for healthy nutrition were focused mainly on their cardioprotective and anticancer activities, while most recently, a lot of studies suggest that flavonoids possess an active role in glucose metabolism and in the reduction of adipose tissue accumulation, rendering their antiobesity potential (Hossain et al., 2016). It has been shown that flavonoids dose-dependently inhibit lipid accumulation (Gamal et al., 2014; Hurt and Wilson, 2012) and it was reported that quercetin exhibits higher antilipase activity (27.4%) than luteolin (17.3%) at 25 mg/mL (Gonzales-Castejon and Rodriguez-Casado, 2011).

Other studies have been focused on catechins and their main dietary source—green tea. A human study has shown that after 3 months, supplementation with extracts standardized to 25% catechins, decreased body weight (4.6%) and waist circumference (4.48%) were observed (Chantre and Lairon, 2002). Green tea catechins have been demonstrated to reduce adipose differentiation and proliferation, lipogenesis, fat mass, body weight, fat absorption, plasma level of triglycerides, free fatty acids, cholesterol, glucose, insulin, and leptine, together with increased β-oxidation and thermogenesis in cell cultures and animal models of obesity (Gonzales-Castejon and Rodriguez-Casado, 2011).

5.4 Flavonoids and Type 2 Diabetes Mellitus

Diabetes mellitus is the most prevalent metabolic syndrome worldwide and arises when insufficient insulin is produced, or when the available insulin does not function properly. Thus, diabetes is characterized by hyperglycemia (elevation of blood sugar levels) resulting in various short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular changes. The long-term manifestation of diabetes can result in development of microvascular complications, such as neuropathy (nerve damage),

nephropathy (renal disease), vision disorders (retinopathy, glaucoma, cataract and corneal diseases), and/or macrovascular complications—heart disease, stroke, and peripheral vascular disease, which can lead to ulcers, gangrene, and amputation (Brahramchari, 2011). In recent years, many studies have emphasized the beneficial effects of flavonoids in the improvement of glucose metabolism, prevention, and management of diabetes and its complications. Very recently, a comprehensive review on antidiabetic properties of dietary flavonoids was published, referring to 217 scientific articles in the field (Vinayagam and Xu, 2015). In this study, following the hypothesis that there is a relationship between diabetes and inflammation, and the potential of flavonoids to protect the body against free radicals, the relationship between flavonoid consumption and reduction of diabetes risk was presented. Based on many cell-line studies and animal models, it has been suggested that all classes of flavonoids possess antidiabetic activity, improve β-cell function and insulin action (Ayepola et al., 2014), improve blood glucose management, increase insulin sensitivity and glucose utilization, increase functional availability of antioxidants, and decrease cholesterol synthesis and triglycerides levels (Nicolle et al., 2011; Vinayagam and Xu, 2015). Following this concept, new functional foods have been developed, with a positive role in maintaining blood glucose levels, glucose uptakes, insulin secretion, and modulation of the immune system (Li et al., 2015).

5.5 Flavonoids and Alzheimer's Disease

There are indications that the antioxidant activity and vasodilatory actions of flavonoids have a beneficial effect against the development of a number of cognitive degenerative diseases associated with aging. Evidence suggests that flavonoids are effective in retarding age-related declines in neurocognitive performance. This positive effect is attributed to their ability to interact with brain cells and the molecular architecture that is associated with memory, and to reduce neuronal loss due to neurodegenerative processes (Spencer, 2010; Vauzour, 2012). A typical example is Alzheimer's disease, which is the most frequent cause of dementia. According to the present state of knowledge, Alzheimer's disease is an irreversible condition; therefore, it is important to find nutrition factors that could postpone or work for healthy management of the disease. An epidemiological study of 1640 subjects revealed that dietary intake of flavonoids is associated with better cognitive function at baseline and leads to favorable development of cognitive performance over a period of 10 years (Letenneur et al., 2007). The positive effect of flavonoid-rich foods (e.g., green tea, blueberry, and cocoa) seems to be mediated by the direct interactions of absorbed flavonoids and their metabolites with a number of cellular and molecular targets (Williams and Spencer, 2012). Although epidemiological and animal studies have lent some support to the neurocognitive potential of flavonoids, human intervention results are less clear. For instance, a recent acute human study demonstrated that cocoa flavonols were able to improve working memory and attention (Field et al., 2011), but at the same time, chronic intervention studies also using cocoa products

showed no significant effects on cognitive performance (Crews et al., 2008). In summary, the consumption of flavonoid-rich foods, such as berries and cocoa, throughout life, holds a potential to limit neurodegeneration and to prevent or reverse age-dependent deterioration of cognitive performance, but the pharmaceutical potential of flavonoids is still to be proven in randomized controlled clinical trials (Nehlig, 2013; Vauzour, 2014).

6 Flavonoid Content and Antioxidant Activity of Selected Bulgarian Plant Foods

A literature review shows that foods of plant origin contain representatives of six major groups of flavonoids—flavonols, flavones, flavan-3-ones, flavanones, anthocyanins, and isoflavonoids. Of these catechins, flavonols and flavones are the most widely distributed in foods and are subjected to intense research (Kumar and Pandey, 2013). Fig. 7.6 represents the chemical structure of the main representatives of catechins, flavonols, and flavones present in fruits and vegetables.

Information about the composition of foods is the basis of all nutritional programs. The development of nutrition science has introduced higher demands for the quality of food composition data to meet the needs of researchers for establishing the relationships between nutrients, foods, diets, and health.

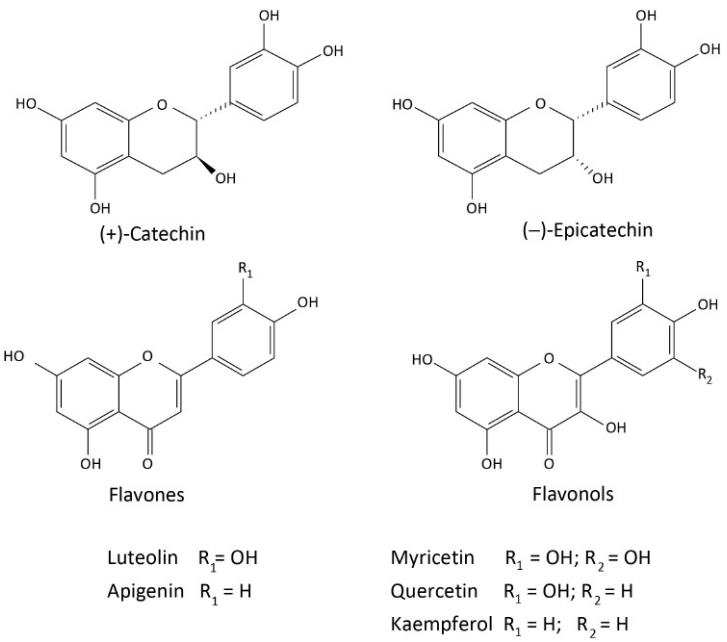


Figure 7.6: Chemical Structure of the Principal Representatives of Catechins, Flavonols, and Flavones.

The first assessment of flavonoids' dietary intake was made by Kühnau in the USA, calculating the total flavonols and flavone-aglycones intake to 115 mg/day (Kühnau, 1976). In 1992, referring to our own accurate studies on the content of the basic flavonols—quercetin, kaempferol, myricetin, and major flavones apigenin and luteolin—in the most often consumed foods in the Netherlands, Hertog et al. evaluated the average daily intake of flavonoids to 23 mg/day (Hertog et al., 1992). In 2002, in the USA, Sampson et al. (2000) established an average daily intake of flavonols and flavones of 20–22 mg/day. The first table on the contents of flavonoids in foods (USDA Database for the Flavonoid Content of Selected Foods) was published in March 2003, prepared by the Department of Agriculture of the United States. The table includes data about the content of aglycones of flavonols, flavones, flavanones, flavan-3-ols (catechins), and anthocyanidins and data are presented in mg/100 g product. The results in the table represent aggregated data, based on the published information in scientific journals. The latest edition of the database was in 2014—USDA Database for the Flavonoid Content of Selected Foods, Release 3.1, May 2014 (Bhagwat et al., 2014). Although some foods, such as onions, are well studied, there is insufficient evidence for the content of flavonols in others, such as cornel cherries. The database shows that differences between maximum and minimum values for flavonoid content could vary significantly. For example, spinach has been reported with a minimum value for quercetin of 0.00 mg/100 g and a maximum value of 27.22 mg/100 g. Such a large variation could be due to biological differences between different varieties of the same species, different cultivation conditions, but also due to incorrect identification of individual flavonoids, as well as differences in analytical procedures or sampling.

The results shown in Tables 7.1 and 7.2 on the content of catechins, flavonols, and flavones in Bulgarian fruits and vegetables are based on the authors' original analytical determinations. High performance liquid chromatography was used for the determination of catechins, flavones, and flavonols. Fluorescence detection was used for the quantification of catechins, and UV-VIS detection with diode-array detection for flavonols and flavones (Tsanova-Savova and Ribarova, 2013; Tsanova-Savova et al., 2005). Original analytical data are presented for 17 types of fruit and vegetables from 6 fruit and 8 vegetable botanical families, among them is a numerous unpublished one. For the first time, a comprehensive compilation of the flavonoid content of Bulgarian fruits and vegetables is presented, based on HPLC analysis of catechins, flavonols, and flavones. It is well known that flavonoids contribute to the antioxidant activity of plants. Therefore, this chapter includes data for the antioxidant activity of the investigated fruits and vegetables, as well. The results reported on antioxidant activity of fruits and vegetables grown in Bulgaria are performed in the Laboratory of Biologically Active Substances at the Institute of Organic Chemistry with the Centre of Phytochemistry at the Bulgarian Academy of Sciences (Číž et al., 2010; Denev et al., 2013). Results are obtained by the oxygen radical absorbance capacity (ORAC) method, in which the inhibition time and inhibition degree are measured as the oxidation reaction goes to completion (Ou et al., 2001).

Table 7.1: Content of catechins and flavonols, and antioxidant activity of Bulgarian fruits.

Fruits	*n*	Catechins		Flavonols			ORAC
		(+)-Catehin	(−)-Epicatechin	Myricetin	Quercetin	Kaempferol	ORAC
		mg/100 g Fresh Weight (Min–Max Value)[a]					μmol TE/g Fresh Weight ± SD
Apple Golden delicious	7	0.52 (0.21–0.89)	3.66 (3.14–4.45)	nd	1.39 (9.5–2.03)	nd	13.8 ± 2.6
Golden delicious (without skin)	7	0.42 (0.11–0.81)	3.00 (2.35–4.00)	nd	nd	nd	nm
Red delicious	7	1.49 (1.08–2.13)	7.39 (6.04–8.85)	nd	1.59 (0.72–2.49)	nd	nm
Red delicious (without skin)	7	0.77 (0.38–0.90)	3.40 (2.13–4.56)	nd	nd	nd	nm
Apricot	5	6.12 (2.46–12.32)	8.34 (6.54–10.25)	nd	3.41 (2.47–4.53)	nd	7.2 ± 1.0
Blackberry	3	1.90 (1.06–2.72)	3.79 (2.43–5.27)	nd	2.70 (2.41–3.17)	0.32 (0.07–0.78)	74.2 ± 3.5
Blueberry	3	2.10 (1.62–2.72)	4.31 (3.09–5.27)	4.32 (3.67–53.3)	9.92 (9.59–10.5)	nd	98.8 ± 7.1
Cherry	5	1.57 (0.92–2.08)	4.79 (1.84–9.03)	nd	2.52 (13.5–3.27)	nd	25.8 ± 1.2
Cornel cherry	3	nd	nd	nd	2.49 (1.95–3.32)	0.47 (0.07–1.16)	49.0 ± 3.5
Grapes, red	7	10.83 (4.55–16.26)	8.70 (2.85–12.56)	0.35 (0.11–0.54)	2.32 (1.90–3.15)	0.11 (0.07–0.32)	26.8 ± 3.4
Grapes, white	7	5.89 (1.42–12.56)	2.78 (0.79–6.11)	0.14 (nd–0.28)	1.56 (0.78–2.39)	0.26 (0.07–0.78)	6.3 ± 1.3
Fig	3	0.43 (0.23–0.60)	0.15 (0.01–0.43)	nd	0.87 (0.24–1.31)	nd	13.6 ± 1.6
Peach	5	2.94 (0.56–2.68)	0.04 (0.01–0.11)	nd	nd	nd	6.2 ± 1.5
Pear	5	0.37 (0.11–0.80)	4.70 (2.09–8.12)	ng	0.59 (0.18–0.89)	nd	nm
Pear without skin	5	0.08 (0.01–0.20)	0.97 (0.54–1.55)	nd	nd	nd	nm
Plum	5	3.31 (1.90–4.98)	0.42 (0.07–0.78)	nd	2.34 (0.82–4.12)	nd	10.8 ± 1.1
Raspberry	5	1.36 (0.61–2.43)	3.67 (1.83–5.02)	nd	1.60 (1.18–2.01)	nd	38.9 ± 2.0
Sour cherry	5	0.30 (0.18–0.41)	0.69 (0.53–0.89)	nd	1.08 (0.32–2.24)	nd	58.6 ± 5.8
Strawberry	5	3.11 (0.52–5.67)	0.18 (0.01–0.68)	nd	1.02 (0.53–1.61)	1.30 (0.76–1.98)	47.2 ± 3.1

nd, Nondetected; nm, not measured; SD, standard deviation.

[a]Minimal and maximal value found in fruits.

Table 7.2: Content of flavonols and flavones, and antioxidant activity of Bulgarian vegetables.

		Flavonols		Flavones		
		Quercetin	Kaempferol	Luteolin	Apigenin	ORAC
Vegetables	*n*	mg/100 g Fresh Weight (Min–Max Value)[a]				μmol TE/g Fresh Weight ± SD
Broccoli	3	2.94 (2.05–3.79)	5.08 (4.02–6.50)	nd	nd	16.1 ± 1.2
Carrot	5	nd	nd	0.88 (0.31–1.43)	nd	4.8 ± 1.1
Celery, leaves	3	nd	nd	22.89 (18.72–25.04)	15.24 (12.42–17.33)	113.5 ± 6.1
Celery, root	5	nd	nd	1.69 (1.17–2.17)	2.95 (2.01–3.83)	15.3 ± 1.2
Cabbage, white	5	0.13 (0.03–0.25)	nd	nd	nd	nm
Cabbage, red	5	0.89 (0.35–1.71)	nd	nd	nd	nm
Dill	3	40.30 (36.74–43.92)	1.79 (1.58–1.97)	nd	nd	10.5 ± 1.1
Green beans	5	2.13 (1.11–3.05)	nd	nd	nd	14.5 ± 1.2
Okra, gumbo	3	20.03 (16.46–23.50)	nd	nd	nd	14.6 ± 0.8
Lettuce	5	15.39 (6.63–27.01)	nd	2.56 (0.07–5.50)	nd	nm
Leek	5	nd	0.83 (0.58–1.42)	nd	nd	12.6 ± 0.8
Onion, spring	5	10.31 (7.56–15.84)	1.46 (0.20–2.71)	nd	nd	14.7 ± 1.5
Onion, red	5	45.25 (26.47–83.22)	nd	nd	nd	24.1 ± 1.2
Onion, white	7	20.41 (9.19–29.28)	nd	nd	nd	25.9 ± 1.1
Parsley	3	nd	nd	nd	74.79 (63.99–84.20)	108.6 ± 13.1
Pepper, green	7	0.46 (0.21–0.79)	nd	nd	nd	nm
Pepper "Kapia," red	7	1.49 (0.71–3.03)	nd	0.69 (0.23–1.38)	nd	9.3 ± 0.9
Pepper "Kapia," green	7	10.27 (4.92–19.49)	nd	2.79 (1.19–5.42)	nd	5.6 ± 0.3
Radish	5	nd	1.35 (0.93–1.78)	nd	nd	23.6 ± 1.7
Tomato	7	1.42 (0.78–2.59)	0.19 (0.07–0.44)	nd	nd	5.4 ± 0.3

nd, Non detected; nm, not measured; SD, standard deviation.

[a]Minimal and maximal value found in vegetables.

The accumulation of biologically active substances in plants depends on many factors including cultivar, climate, fertilization, irrigation, sun exposure, and so on. Ou et al. (2002) reported variable results for the antioxidant activity, even for samples from one species, depending on the variety, place of origin, and harvest time. Therefore, it is of a particular interest to evaluate the phytochemical composition and antioxidant activity of local natural products, as they are the most consumed by the local population. The presented results are part of a long-term study, carried out for the development of a database with antioxidant capacities of Bulgarian plant foods. The development of such a database will identify the major contributors to the antioxidant capacity of our daily diet. In literature, similar databases with antioxidant activity have been reported for plant foods in the United States (Wu et al., 2004) and fruits from South America (Speisky et al., 2012).

6.1 Flavonoid Composition and Antioxidant Activity of Bulgarian fruits

6.1.1 Flavonoid composition

Table 7.1 presents data on the minimum and maximum value (in mg/100 g fresh weight of edible portion), which outlines the interval of the results for catechin and flavonol content in various fruits. The analyses showed that usually (–)-epicatechin content in fruits is higher than that of (+)-catechin, except for peaches, plums, strawberries, grapes, and figs. The data shows that quercetin is the most common flavonol in fruits, while myricetin and kaempferol are only found in certain fruit species.

6.1.2 Family Rosaceae

Data shows that fruits of Rosaceae family are rich in catechin and epicatechin. Analysis of apples and pears before and after removal of the fruit skin shows that catechins are concentrated mainly in the skin, but significant quantities are found in the core, as well. Results indicate that fruits of the family Rosaceae do not contain myricetin. The only fruit species of this botanical family in which kaempferol was found are blackberries and strawberries.

The results for the most widely grown and consumed apple varieties—Golden Delicious and Red Delicious—showed that variety determines the different amounts of catechin and epicatechin in fruits. For example, Red Delicious apples with skin are about two times richer in catechins than Golden Delicious with skin. The amount of catechins in Bulgarian Golden Delicious apples (0.52–3.66 mg/100 g) is very close to the results reported by Arts et al. (2000) (5.3–7.42 mg/100 g) and De Pascual-Teresa et al. (1998) (0.15–2.00 mg/100 g). The quercetin content in both varieties is similar: 1.39 and 1.59 mg/100 g, respectively, for Golden Delicious and Red Delicious. Previous research on various apple varieties has established that apples accumulate quercetin only in the skin and, unlike catechins, flavonols are not found in the core of the fruit (Justesen et al., 1998).

The removal of the skin from pears dramatically affects the content of (+)–catechin and (–)-epicatechin, decreasing them from 0.37 to 0.08 mg/100 g and from 4.70 to 0.97 mg/100 g, respectively. The amount of epicatechin in pears is close to that in apples, but the content of catechin is lower. Our results for quercetin content in five pear samples—0.59 mg/100 g (0.11–0.80 mg/100 g)—are within the range of data included in the USDA Database of Flavonoids Content of Selected Foods—0.84 mg/100 g (0.00–3.40 mg/100 g) (Bhagwat et al., 2014).

Our results clearly indicate that Bulgarian apricots are very rich source of (+)-catechin with mean values of 6.12 mg/100 g (maximum value of 12.32 mg/100 g) and (–)-epicatechin with an average value of 8.34 mg/100 g (maximum value of 10.25 mg/100 g). These values are among the highest reported in literature (Bhagwat et al., 2014). The average quercetin content in apricots (3.41 mg/100 g, $n = 5$) was significantly higher ($P < 0.05$) than that reported in USDA Database, 2014 (1.36 mg/100 g, $n = 34$).

Unlike the other fruits, the content of (–)-epicatechin in peaches is very low with an average value of 1.23 mg/100 g and the results for (+)-catechin and (–)-epicatechin content are similar to the data (Arts et al., 2000; De Pascual-Teresa et al., 1998). Furthermore, our results indicate that myricetin, quercetin, and kaempferol are not detected in peaches.

Plums contain significant amounts of catechin and low levels of epicatechin and are among the few fruits with higher quantity of (+)-catechin compared to (–)-epicatechin.

In Bulgarian cherries, the content for epicatechin varies widely (1.84–9.06 mg/100 g). This is probably because of the differences between early cherry varieties that bear fruit in May and those bearing fruit in June.

Our results show that the amounts of quercetin in Bulgarian plums and cherries are significantly higher than the reported (Bhagwat et al., 2014). The differences could be explained on the one hand with the scarcity of data and on the other with differences in cultivated varieties of fruits in different countries. The highlighting of such differences in the composition of foodstuffs justifies the development of a national database for the content of nutrients and bioactive compounds in food. The results indicate that sour cherries have approximately 2.5 times less quercetin than cherries. We have found that in sour cherries the amounts of catechins were very low with 0.30 and 0.69 mg/100 g of catechin and epicatechin, respectively.

The results for raspberries, blackberries, and strawberries indicate that the raspberries, and especially blackberries, have a much lower content of epicatechin in comparison to the data of Arts et al. (2000), but similar to the results of De Pascual-Teresa et al. (1998). The difference could be due to the study of fruit at different ripeness stages, as it is known that flavonols in strawberries and black currants accumulate in large quantities during early embryonic development, after which they decrease continuously until reaching a constant value in their mature stage (Aghofack-Nguemezi and Schwab, 2014).

6.1.3 Family Ericacea

Blueberries were the only representative of the *Ericaceae* family in our study. They demonstrate very high levels of catechins compared to literature data, wherein the maximum values for catechin and epicatechin are 0.81 and 1.11 mg/100 g, respectively (Arts et al., 2000; De Pascual-Teresa et al., 1998). Our results for (+)–catechin are more than twice as high, and for (–)-epicatechin, about four times higher than those quoted. These differences might be due to the fact that blueberries in Bulgaria are entirely wild species, while in the aforementioned studies cultivated varieties were analyzed. The fact that blueberries in Bulgaria are organically grown could explain the large amount of their catechin content, since there is evidence that during environmental stress, plants build up flavonoids in larger amounts (Mierziak et al., 2014).

Blueberries are also the richest source of myricetin among the investigated fruits with an average value of 4.31 mg/100 g (3.67–5.33 mg/100 g). The amounts of quercetin in the examined blueberries were also very high (average value of 9.92 mg/100 g). When comparing the data obtained in the literature, the amounts of flavonols in Bulgarian blueberries are comparable to those reported in the USDA database (Bhagwat et al., 2014), but are higher than the amounts in cultivated varieties (7.67 mg/100 g).

6.1.4 Family Vitaceae

The results in Table 7.1 indicate that the average value of catechin and epicatechin in red grapes is 10.08 and 8.70 mg/100 g, respectively. This shows that red grapes are the richest source of catechins among Bulgarian fruit. The results for the content of catechin (5.89 mg/100 g) and epicatechin (2.78 mg/100 g) in white grapes are the highest currently reported. Our results are significantly higher than those included in the USDA database for Flavonoids Composition of Selected Foods ($P < 0.05$) (Bhagwat et al., 2014). Besides, grapes are the only fruits in which all three flavonol representatives can be detected. The results of the analysis of red and white grapes show that the mean values for myricetin content are 0.35 and 0.14 mg/100 g, the values for quercetin are 2.32 and 1.56 mg/100 g, and the values for kaempferol are 0.11 and 0.26 mg/100 g. The available databases do not indicate the presence of kaempferol in grapes, while a recent study reported very low quantities (Bhagwat et al., 2014).

6.1.5 Family Moraceae

The data shows that figs are very poor to catechins compared to other fruits analyzed, which confirms the results of De Pascual-Teresa et al. (1998). Figs contain only quercetin, which varies in the range of 0.24–1.32 mg/100 g with average value of 0.87 mg/100 g.

6.1.6 Family Cornaceae

The results show that cornels are a relatively rich source of quercetin (2.49 mg/100 g edible portion) and one of the few fruits containing kaempferol. The content of quercetin is similar to that in plums, cherries, blackberries, and black grapes. There was no comparable information about the content of flavonols in cornels (*Cornaceae*) and figs (*Moraceae*).

6.1.7 Antioxidant activity

Fresh fruits are a good source of antioxidants and their use in human nutrition is of fundamental importance. It is important to recognize which fruits have the highest antioxidant activity and to promote their regular consumption among the population. As it is seen from Table 7.1, the total antioxidant activity varies considerably among the investigated fruits. For example, based on fresh weight, blueberries showed the highest antioxidant capacity (98.8 µmol TE/g), followed by black currants with (96.0 µmol TE/g). There are many papers attempting to rank the antioxidant properties of different plant materials via different methods (Pellegrini et al., 2003) including oxygen radical absorbance capacity (Ou et al., 2002; Wu et al., 2004). When comparing our results with other published results it is seen that Bulgarian fruits reveal different antioxidant properties. Our results show 98.8 and 47.2 µmol TE/g for strawberries and blueberries are 18.5 and 37% higher than the results of Wu et al. (2004) for the same plant species. In one of the first attempts to quantify daily antioxidant needs, Prior et al. (2005), demonstrated that consumption of certain plant foods is associated with increased plasma antioxidant capacity. On the other hand, the consumption of energy-rich macronutrients containing no antioxidants led to a decline in plasma antioxidant capacity. The authors estimated that approximately 5,000–15,000 ORAC units per day are necessary to cover human daily antioxidant needs. This means that the consumption of about 50 g of blueberries will cover our daily antioxidant needs, whereas we need about 700 g of apricots for the same daily antioxidant protection.

6.2 Flavonoid Composition and Antioxidant Activity of Bulgarian Vegetables

Flavonoid composition: A review of the literature suggests that catechins cannot be detected in vegetable crops, with only trace amounts of (+)-catechin and (–)-epicatechin in legumes of the family Papilionaceous. Results for the content of flavonols quercetin and kaempferol and of the flavones apigenin and luteolin in fresh vegetables are presented in Table 7.2. It does not include data on myricetin as it is not detected in vegetables. The results are presented in mg/100 g fresh weight of edible portion.

As presented in Table 7.2, the flavones are less abundant than flavonols. Their presence is limited only in vegetable crops from three botanical families: Solanaceae, Asteraceae,

and Umbelliferae. Kaempferol is found in a limited number of vegetables, ranging from 0.10 mg/100 g in tomatoes to 5.08 mg/100 g in broccoli.

6.2.1 Family Solanaceae

Tomatoes and peppers were analyzed as representatives of the family Solanaceae. The results revealed that the average content of quercetin in tomatoes was 1.42 mg/100 g, which is significantly higher than the results of Stewart et al., 2000 (0.67 ± 0.44 mg/100 g, $n = 13$) and is comparable to the data presented by Justesen et al. (1998) (1.40 ± 0.8 mg/100 g, $n = 5$). It seems that the cultivation of Bulgarian tomato varieties in combination with microclimate conditions is favorable for the accumulation of high amounts of flavonols.

Table 7.2 shows the results for quercetin content in peppers from two different varieties—green pepper Syvria and green and red Kapiya peppers. The results show that green Syvria peppers are characterized by low levels of quercetin that reach a maximum value of 0.74 mg/100 g and average value of 0.46 mg/100 g. In technological ripeness, Kapiya peppers are dark green and when fully ripe they become red. Results for the sweet Kapiya pepper show that the average quercetin content of the red specimen is 1.49 mg/100 g, while green Kapiya peppers are extremely rich in quercetin with an average of 10.27 mg/100 g. This fact can be explained by the general trend for flavonoids to be synthesized in large quantities in the early stages of fruit development, followed by decreasing quantities in the course of ripening. Luteolin in green Kapiya peppers has an average value of 279 mg/100 g and decreases to 0.69 mg/100 g at the botanical maturity of the vegetable.

6.2.2 Family Asteraceae

Lettuce was analyzed as a representative of Asteraceae family. The results show that lettuce is one of the food products with higher contents of quercetin (15.39 mg/100 g). Luteolin was detected only in three of five lettuce samples tested, in amounts up to 5.50 mg/100 g. A literature review shows that Hertog et al. (1992) failed to detect luteolin in lettuce. Conversely, DuPont et al. (2000) isolated and identified glycosides of luteolin in lettuce. In the latter publication, the identification of luteolin-7-glucoside has been explicitly confirmed by the means of mass spectrometry and nuclear magnetic resonance. The contradictory data could be explained by the varying luteolin content in various lettuce samples; for example, in some cases the amount is so low that it is less than the limit of detection for the utilized method. These results are of interest and require further study.

6.2.3 Family Umbelliferae

The representatives of this family are the vegetable species with the highest amounts of flavones and they are the only crops in which apigenin is detected. Luteolin was detected in all carrot samples, in amounts ranging from 0.31 to 1.43 mg/100 g. According to the literature

data, carrot leaves are rich in glycosides of luteolin and apigenin (Bhagwat et al., 2014), but in their roots flavones were not detected in quantities greater than 1.1 mg/100 g (Bhagwat et al., 2014).

Celery and parsley are among the main sources of flavones in the Bulgarian diet. Celery leaves are extremely rich in luteolin and apigenin. Their level decreases in the roots, but remains significant (Table 7.2). Parsley itself contains only apigenin, but in very significant amounts, in that only one gram yields about 0.7 mg of apigenin.

Carrots, celery, and parsley do not contain flavonols, but unlike them, fennel is rich in quercetin with an average of 40.3 mg/100 g. The content of kaempferol was significantly lower (1.79 mg/100 g). The data obtained confirms the results of Justesen et al. (1998), who report that dill is very rich in quercetin and even its addition to various dishes in small quantities influences the intake of flavonols.

The results presented in Table 7.2 show that flavones were detected in only five of the studied vegetables. In parsley, celery, and carrots only flavones are available, while in pepper and lettuce flavones and flavonols were detected. In parsley, the amount of flavones was so high (74.79 mg/100 g), that it exceeds several times the level of flavonols and flavones combined in all other vegetables. The group containing celery leaves (47.93 mg/100 g flavanones), red onions (45.25 mg/100 g flavonols), and dill (42.09 mg/100 g flavonols) contains approximately 30.0 mg/100 g less flavonols than parsley. The total content of flavonols and flavones in white onions (20.56 mg/100 g), okra (20.04 mg/100 g), and lettuce (17.48 mg/100 g) is within the range of 20.0–15.0 mg/100 g, while their amounts in tomato, radish, carrot, leek, and red and white cabbage is lower than 0.2 mg/100 g fresh weight.

6.2.4 Family Liliaceae

Leek and three varieties of onions: white, red, and green (fresh) were investigated. Analysis was conducted separately on the feathers, the white part of the stem, and on the whole vegetable to study the accumulation of flavonols in different parts of the plant in more detail.

Red onions contain the highest amounts of quercetin among all tested vegetables with an average of 45.25 mg/100 g and maximum of 83.22 mg/100 g. In onions, similar to other fruits and vegetables, quercetin is mostly accumulated in the outer layers of the bulb.

Unlike white and red onions, spring onions are much less studied. Justesen et al. (1998), presented data on the content of flavonols in samples of spring onions. We have found that spring onion leaves contains quercetin (3.02–6.11 mg/100 g) and kaempferol (2.74–5.23 mg/100 g) and the white part of the onion has large amounts of quercetin (11.02–23.05 mg/100 g). The analysis of the whole plant reveals that it has average quercetin and kaempferol content within the values for the aforementioned samples.

Similar to the results for green onions, kaempferol in leeks is detected only in the green part of the stem, but unlike fresh onions, quercetin in leeks is detected neither in the green nor in the white part of the plant stem.

6.2.5 Family Cruciferae

The content of flavonols in the species of this family is quite varying. Flavonols were not detected in cauliflower, red beets, or turnips. In red and white cabbage, the amount of quercetin is very low—0.13 and 0.89 mg/100 g, respectively. The results for broccoli and Brussels sprouts are of particular interest, since they are among the main sources of kaempferol in the diet. Only in broccoli is the kaempferol concentration higher than that of quercetin: 5.08 and 2.94 mg/100 g, respectively. The amount of quercetin and kaempferol are almost the same in Brussels sprouts: 2.63 and 2.66 mg/100 g, respectively. In radishes, the measured content of kaempferol was an average of 1.35 mg/100 g and quercetin was not detected.

6.2.6 Family Leguminosae

Legumes, especially green beans, are a staple food in many regions of the world. As such, they are researched intensively to determine their content of myricetin, quercetin, and kaempferol. We investigated the amount of quercetin in two types of green beans for fresh consumption: flat green pods and flat yellow pods. Kaempferol was not detected, while the average quercetin content is 2.13 mg/100 g, which corresponds to literature data: 27.3 mg/kg (Bhagwat et al., 2014).

6.2.7 Family Malvaceae

Results from the analysis of three samples of *Hibiscus esculentus* show that okra (gumbo) is extremely rich in quercetin (16.46–23.50 mg/100 g). We could not find comparable literature data for the content of flavonols in this vegetable. Okra is used in the preparation of various dishes in Bulgaria and is a particular source of flavonols in the national diet.

6.2.8 Antioxidant activity

Because of their high consumption, vegetables are among the major antioxidant sources in our daily diet. Therefore, the estimation of daily antioxidant capacity intake from these foods is of great value. Vegetables are known to possess a variety of antioxidant effects and properties. Flavonols (such as quercetin, kaempferol, and myricetin) and flavones (e.g., apigenin, luteolin) in plant materials are closely associated with their antioxidant function, mainly due to their redox properties exerted by various possible mechanisms: free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity. There are many papers attempting to rank the antioxidant properties of different plant materials using different methods including ORAC (Ou et al., 2002; Wu et al., 2004)

but the results given later are the first reporting antioxidant activity of Bulgarian vegetables. The total polyphenol content of these selected vegetables was measured as well, so as to evaluate its contribution to their total antioxidant function. Results are shown in Table 7.2 and expressed as means ± standard deviations (Číž et al., 2010). Using the Oxygen Radical Absorbance Capacity assay we obtained a hierarchy of antioxidant capacity ranging from 113.0 to 1.2 μmol TE/g of fresh weight and the rank order was as follows: celery leaves > parsley > chilli pepper > radish > broccoli > celery root > spring onion > okra (gumbo) > green bean > dill > red pepper > green pepper > tomato > carrot.

To visualise the results, a graphical form is presented in Tables 7.1 and 7.2, which allows for the rapid assessment of the richest food sources of total flavonoids and the raw materials with the highest ORAC values. Additionally, data for the total polyphenol content of fruits (Fig. 7.7) and vegetables (Fig. 7.8) is added.

According to the results presented in Fig. 7.7, there was a direct relationship between the total phenolic content and ORAC antioxidant activity of fruits with correlation coefficient $r = 0.95$, and ORAC values correlate better with the total polyphenol content than with the flavonoid content. This could be explained by the presence of other polyphenol compounds in samples, as it is evident from the graph that flavonoids represent only a small portion of the polyphenols present in fruit samples.

Fig. 7.8 presents the results for the overall content of flavonols and flavones in selected Bulgarian vegetables, as well as the results for their total polyphenol content and their ORAC antioxidant activity.

The results in Fig. 7.8 show that parsley and celery leaves are the richest sources of flavonoids (flavones and flavonols) and possess the highest antioxidant activity among the investigated vegetables. Furthermore, the amount of total polyphenols in these leafy condiments is about fivefold higher than in the other vegetables. An interesting case here is dill, which despite having high amounts of flavonoids and total polyphenols revealed moderate antioxidant activity, comparable to that of green beans and celery roots. Similarly to results for fruits, it is evident that flavonoids represent just a small portion of the phenolic compounds present in vegetables.

The present study is the first to present consolidated analytical data of flavonoid content of Bulgarian fruits and vegetables based on individual HPLC determination of catechins, flavonols, and flavones, along with original data for their total polyphenol content and ORAC antioxidant activity. The present set of data is of great importance, not only for nutritional science, but for the characterization of bioactive compounds in plant foods as well. The results presented in Figs. 7.7 and 7.8 clearly show the necessity of combining data from different sources, obtained via different analytical methods and procedures in order to better understand and interpret their integrity in the dietary recommendation for healthy nutrition.

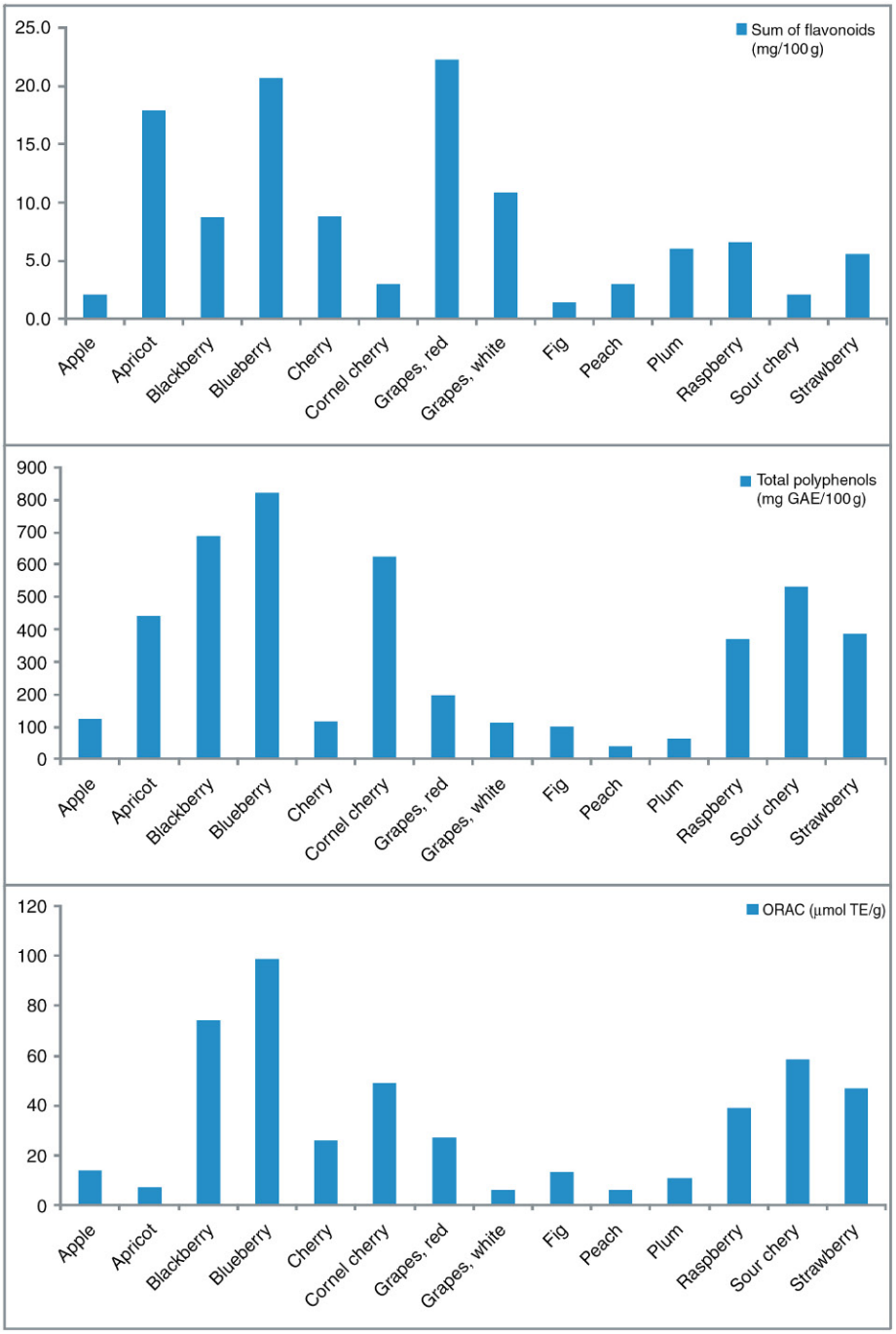


Figure 7.7: Sum of Flavonoids (Catechins and Flavonols), Total Polyphenol Content and ORAC Antioxidant Activity of Bulgarian Fruits.

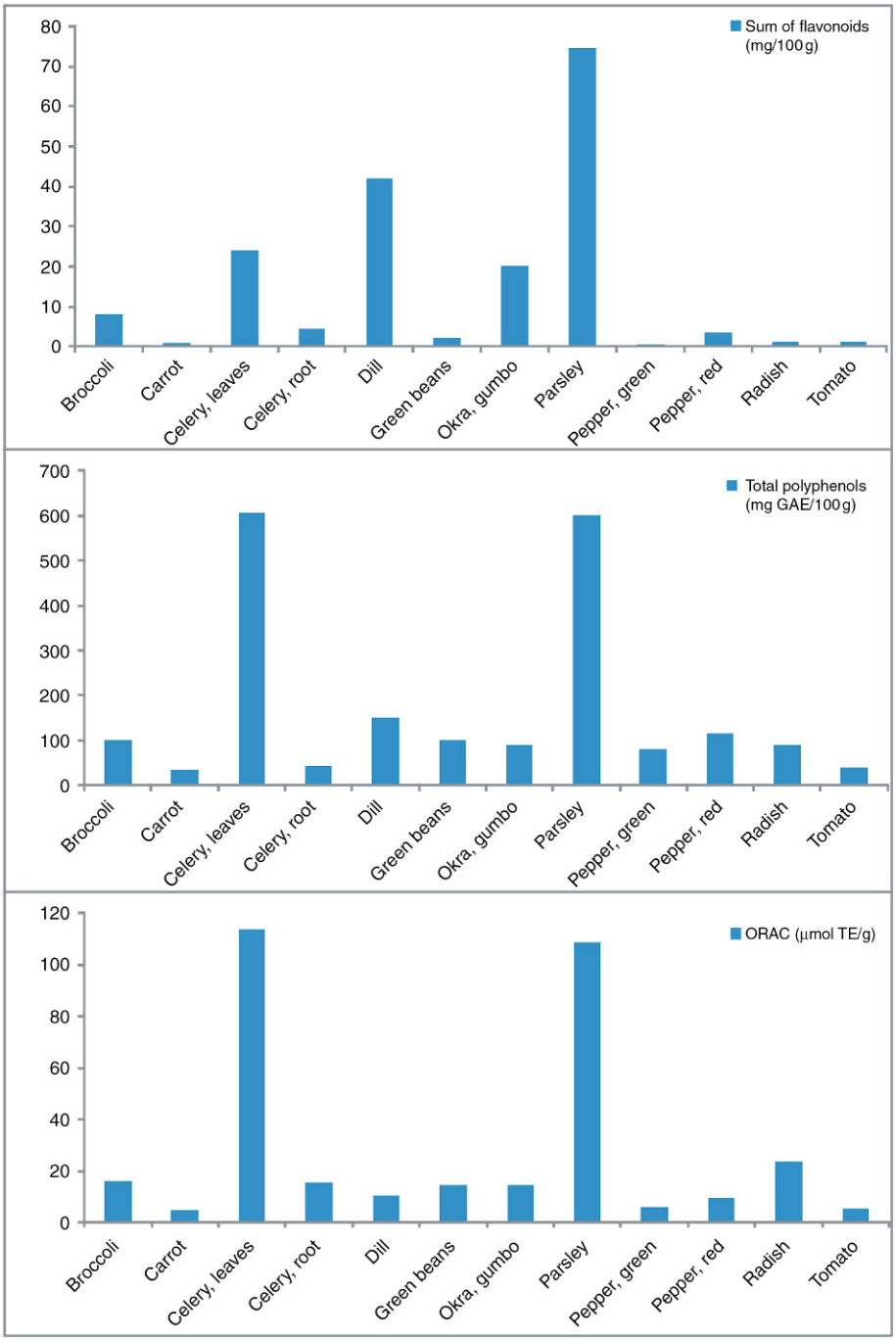


Figure 7.8: Sum of Flavonoids (Flavonols and Flavones), Total Polyphenol Content and ORAC Antioxidant Activity of Bulgarian Vegetables.

7 Conclusions

Flavonoids are potent antioxidants, acting along with antioxidant vitamins, protecting the human body against reactive oxygen species. Their beneficial role for human health was confirmed during the last 20 years, since the first study of their potential protective effect against cardiovascular disease was described. Flavonoid consumption might also be associated with reduction of risk for the development of some forms of cancer, obesity, and diabetes, and has shown improvement of cognitive function in those with Alzheimer's disease.

The present results for the flavonoid content, ORAC antioxidant activity, and total polyphenols content show that leafy vegetables, such as parsley, dill, and celery leaves are among the best sources of polyphenols and antioxidants in the Bulgarian diet. The obtained results are a useful tool for medical professionals to promote the consumption of plant foods with high antioxidant activity as a part of a healthy lifestyle.

Flavonoids are one of the top research areas in food and nutrition science concerning their bioactivity and potential to promote health and to reduce the incidences of coronary heart disease, diabetes, cancer, and other chronic diseases. Recent epidemiological studies have confirmed and enhanced the scope of diseases and pathophysiological conditions where flavonoids have displayed certain positive health effects, thus challenging science to clarify all aspects associated with their bioactivity.

Although a great number of flavonoid representatives have been identified, studies continue searching for new bioactive components in the broad variety of plant species. Those data are of vital importance for the compilation of national/regional food composition databases that are used in global nutritional policy and information exchange. Besides that, they are important for the characterization and identification of existing biodiversity. The current stage of research implements unified sampling approaches and strives to use more and more perfect analytical equipment, providing high precision level and very good reproducibility, guaranteeing significant results.

Large scientific potential is focused also on the assessment of bioaccessibility, bioavailability, and bioactivity of flavonoids that could be modulated on the basis of impact of various factors along the gastrointestinal tract with special emphasis on microbiota. The effect on the processes of digestion, absorption, and metabolism cause certain changes in flavonoid bioactivity. Numerous further studies are necessary in this aspect in order to establish the action specifics of the various factors and clarification of the biochemical mechanisms associated with the modifications of flavonoid bioefficacy.

From a bioengineering point of view, it should be noted that the current knowledge on flavonoids also covers the development of new technologies using various nanoparticles and nanoemulsions for the protection of native flavonoid activity, preventing the impact of relevant gastrointestinal factors.

The content of this chapter unites current scientific evidence with our experience, knowledge, and data on flavonoids, from their identification in the different plant species, through their prevalence in nature, analytical approaches for their determination, their bioaccessibility and bioavailability, determination of their exact composition and content in various foods, description of their activity and the role of new technologies in the preservation of their native power. All those dimensions manifest the significance of the extensive flavonoids issue. The power, assigned by nature through plant food flavonoids is a challenge to nutrition science.

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Further Reading

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