Flavonoids as Key Medicinal Components in Honey

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By

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TABLE OF CONTENTS

PREFACE

Honey is a natural complex mixture of very different chemical ingredients; first of all, different sugars, mainly fructose and glucose, water and other substances that get into honey during its creation, which is produced by the honey bee (*Apis mellifera*) and, regardless of the progress of the industry, cannot be replaced by some production process. Namely, honey consists of over 200 different substances (proteins, free amino acids, vitamins, organic compounds, enzymes, minerals and others) with extremely high nutritional values and specific organoleptic properties. Due to its high nutritional value, pleasant organoleptic properties, and easy digestibility, honey has been an important part of the diet since ancient times, and due to its composition, sweetness and physicochemical properties, it is an ideal substitute for table sugar.

The book “Flavonoids as key medicinal components in honey” provides fundamental knowledge in the field of potentional of medicinal properties of honey and secondary metabolites of flavonoids. The book was written as a result of the author’s many years of scientific research in the field of testing the quality of Bosnian-Herzegovinian honey and its potentially medicinal properties. Numerous published studies examining the medicinal properties of flavonoids were analysed.

It represents one of the first scientific and educational works of this type, which focuses on the medicinal properties of secondary metabolites of flavonoids and their introduction into the human body from honey.

This book is primarily intended for students, undergraduate and postgraduate, in the fields of natural sciences, medicine, pharmacy, agriculture, nutrition, and ecology, but also for all those who are professionally involved in honey production or are interested in research in this field. It is written in such a style that it should be an incentive for further research of medicinal components in honey and bee products from Bosnia and Herzegovina and all over the world.

The book is a very important work for the scientific research and academic community, beekeepers and others.

Be aware of the fact that this book deals with a very complex professional and scientific field, and it should answer the most important questions concerning the medicinal properties of honey based on the content of flavonoids.

The author
Harun Kurtagić

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This book would not have been published without the selfless support of my dear family, my wife, who is lawyer, and my two children, who are students of medicine, for which I remain eternally grateful.

The author
Harun Kurtagić

CHAPTER ONE

INTRODUCTION

Food consists of a large number of known and unknown natural biologically active ingredients, which can contribute to the improvement of human health. Biologically active food ingredients are often defined as natural, non-nutritive ingredients that can benefit human health if consumed adequately. Otherwise, they can have toxic, negative effects on human health. Biologically active substances of a nutritional nature are amino acids, fatty acids, etc., and of a non-nutritive nature are: polyphenols, flavonoids, chlorophyll, isothiocyanates, phytoestrogen glycosides, alkaloids, sterols, carotenoids and others. The connection between man and plants dates back to ancient times. Man primarily used plants for nutrition and later also for treatment, so they became more and more important as a source of biologically active substances. Improving human health has always been the goal of scientific research in the fields of chemistry, biochemistry and medicine. A large number of therapeutic agents used in modern medicine have been obtained by examining the chemical, biological and pharmacological properties of natural products used in traditional medicine around the world (Jašić 2010).

The subject of current research in the world is the medicinal properties of plants, which are important factors that improve people's general health. First, the medicinal property is explained by the presence of secondary metabolites in plants, which can show different biological activities (Harnborne and Herbert 1999). Flavonoids are considered the main bioactive ingredients of many medicinal plants, on which their special antioxidant activity is based. However, due to their poor solubility in water and oil, their use in food and medicine is limited. Flavonoids (red, green, orange, yellow and dark blue pigments) from berries, tropical and citrus fruits, as well as vegetables individually or in combination, act as antioxidants against free radicals and, in this way, significantly reduce the risk of damage to the DNA structure and the occurrence of cancer (Socha et al. 2009).

Compared to some pharmaceutical medicinal substances, phytochemicals have a lower bioactive potential, but due to the fact that they are ingested daily in small amounts through the diet, they can cause a noticeable physiological effect in the long term. Throughout history, almost the only and irreplaceable form of treatment was represented by treatments with plant extracts and compounds isolated from natural sources (Jašić 2010). In addition to their undeniable importance for the pharmaceutical industry, natural plant products are widely used in the production of dietary supplements and functional foods, which, in addition to satisfactory nutritional properties, also exhibit certain pharmacological and physiological effects on human health, which is very important in the prevention of diseases in modern man life.

For this reason, the examination of biological activity and chemical characterization of until now unexamined plant species are of exceptional scientific and practical interest, as they lead to new sources of biologically active substances (Herborne and Herbert 1999; Kaškoniene et al. 2009).

It is believed that free radicals are the cause of many pathological conditions in the body. It has been established that many different diseases result from disruption of cellular functions, that is, damage to the cell itself caused by the action of free oxygen radicals. The body defends itself against free radicals, in addition to its own defence mechanism, with natural antioxidants that are introduced into the body through food (Jašić 2010). The medicinal effect of plants on health is attributed to the high content of various phytochemical compounds, the most abundant of which are phenolic compounds. Phenolic compounds have strong antioxidant and antiradical activity (Heinonen et al. 1998; Herken et al. 2010).

The most important and largest group of polyphenols are flavonoids, which appear in almost all parts of plants. The functions of flavonoids in plants are multiple (Herborne and Herbert 1999; Chen et al. 2004).

The diversity and large number of flavonoid structures are the result of numerous modifications of their basic structures, such as: additional hydroxylation, O-methylation of hydroxyl groups, dimerization, binding of inorganic sulfate and glycolization of hydroxyl groups (formation of O-glycosides) or flavonoid core (formation of C-glycosides). Also, the variety of flavonoids is controlled by the plant's genes, but also the maturity of the plant, the climate and the cultivation method (Herborne and Herbert 1999; Cushnie and Andrew 2005). Flavonols and flavones are of particular importance because they have been found to have antioxidant and free radical scavenging abilities. Flavonoids are attributed to influencing the colour and taste of food. Obviously, these are compounds that have an important role in maintaining and protecting the vital functions of plants and having entered them through food; they have a similar role for other living beings. Because of all the positive properties, these compounds are the subject of research by many scientists (Herborne and Herbert 1999; McKiben and Nicki 2002; Jašić 2010). Epidemiological studies from the beginning of the 90s indicate a correlation between flavonoids and a reduction in the risk of many degenerative diseases. Due to, their great nutritional and therapeutic importance, flavonoids today attract the most attention among phytocompounds. Many studies on the human population indicate the protective effect of flavonoids when it comes to cardiovascular diseases. In the last few tens of years, research has shown that there is a connection between individual structural components and the property of capturing radicals, forming chelate complexes and antioxidant activity (Jašić 2010). The antioxidant activity of flavonoids depends on their molecular structure, i.e. hydroxylation and alkylation of the A and B rings of these compounds, then the location of the free hydroxyl groups on the A ring and the OH group on the C-3 position of the C ring (Figure 1). Flavonoids that have a hydroxyl group in *the ortho* position in the B ring and multiple hydroxylations in the A ring are more effective antioxidants. Glycosylation of one or more OH groups of flavonoids significantly reduces antioxidant activity (Herborne and Herbert 1999). A large number of scientific studies point to the fact that free radicals are the main cause of lipid peroxidation, which represents one of the most significant chemical changes in food products during food processing and storage (Herborne and Herbert 1999; Ferreira et al. 2009; Benjamin and Brian 2010).

Aromatic plants occupy a very important place in these researches, thanks to the biological functions of their secondary biomolecules, primarily terpenoids, and especially essential oils, which represent a mixture of volatile compounds from the class of mono- and sesquiterpenes. In addition to essential oils, aromatic plants are also characterised by the presence of a large number of phenolic compounds such as: phenolic acids, flavonoids, catechins, tannins, etc., which have been proven to possess multiple pharmacological activities, primarily a high antioxidant potential (Soler et al. 1995). Given that honey is a product produced by bees from honey-bearing plants, this food product is the richest in antioxidants, including flavonoids (Chadwick and Joan 2008). Bee honey is one of the safest foods because most dangerous bacteria cannot live in it at all (Mulalić and Grujić 1986; Radić and Grbić 2005). It can rightly be said that honey and other bee products are a gift of nature and the best food product for human consumption.

The bee is one of the wonders of nature. Besides being its most efficient pollinator, honey bees (*Apis mellifera, Linnaeus,* 1758) produce excellent and healthy food that people have been eating for centuries (Benjamin and Brian 2010). There are four main groups of honey bees, but the greatest attention has been given to this species because of its excellent productivity in making honey and pollinating crops. Entomologists, with their advanced technology, have established the existence of more than 20 thousand species of bees in the world that pollinate most of our planet's flora. They feed on nectar as a source of energy, and pollen as a source of protein. There are three castes in the honey bee community: queen, worker and drone (Mulalić and Grujić 1986; McKiben and Nicki 2002; Benjamin and Brian 2010).

Each has a specific function within the community. The worker bee lives 30-45 days in the season, and the queen lives up to seven years and hatches eggs up to three times her weight per day. In the spring, each surviving queen establishes a new social community. The basic products of the bee are: honey, pollen, propolis, royal jelly and bee venom. Man produces many secondary products from bee products in nutrition, pharmaceuticals, cosmetics, etc. (Radić and Grbić 2005). In order to produce honey, the most important bee product, the bee first visits the flowers sucks their nectar and carries it in its bag to the queen bee. That bag is an integral part of the bee's body, an extension of the digestive organ located in the front of the stomach, but completely separate from it. Sugar, as an integral part of the nectar taken from the flowers, changes chemically while it is still in the bee's sac, which represents the first stage in the creation of honey from flower nectar. Before the nectar is processed into honey, the bee removes most of the water from it through the process of evaporation. The honey that bees put in their combs contains a very small amount of water so that it can last almost forever (Mulalić and Grujić 1986; Radić and Grbić 2005; The National Honey Board 2007).

Bees have an innate ability to adapt even to harsh climatic conditions. Their social organization is very efficient and complex, and to some extent, it can be compared to the human social community. Bees are vital to agriculture because they pollinate crops. Understanding bee biology is key to sustaining this industry in the face of problems such as the death of entire colonies. Honey is made from nectar – a watery, sweet liquid found in the flower parts of pollinating plants or other plant parts. Nectar attracts bees, hummingbirds, butterflies and ants who like to suck it up, but bees do a bit more than other insects collect pollen and spread it around, which allows plants to be pollinated. Bees turn collected nectar and pollen into honey with the help of enzymes in their own bodies (Mulalić and Grujić 1986; Benjamin and Brian 2010). Honey is one of the oldest known medicines; even today, in folk medicine, it is used in the treatment of wounds, burns, ulcers and also in the prevention of infections. The main factors that determine the antimicrobial properties of honey are: osmotic effect, acidity, hydrogen peroxide and plant substances, such as flavonoids. The total amount of pollen in honey is one of the criteria for choosing the right honey, because the quality is related to the plants visited by bees. The antioxidant properties of bee products are mainly based on the content of polyphenolic compounds (Namiki 1990; Rice-Evans and Nicholas 1994; Halliwell et al. 1995; Huxley et al. 2005; Cereilo 2008; Dalgić et al. 2011).

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CHAPTER TWO

BIOSYNTHESIS OF FLAVONOIDS AND THEIR PROPERTIES

Natural aromatic compounds are formed during two biosynthetic pathways: the shikimic acid cycle and the acetate-malonate cycle, with the aim of forming the basic structural unit of flavonoids C6–C3–C6. Flavonoids are synthesised from aromatic amino acids (most often phenylalanine, less often tyrosine and tryptophan), and the precursor in the synthesis of aromatic amino acids is shikimic acid (Marais et al. 2006).

Carbohydrate metabolism produces phosphoenolpyruvate (glycolysis) and erythrose-4-phosphate (pentose-phosphate pathway), the condensation of which produces shikimic acid. Aromatic amino acids (phenylalanine, tyrosine and tryptophan) are formed through a series of reactions from shikimic acid, and this biosynthetic pathway is called the shikimic acid cycle. This cycle produces compounds of the C6-C3 type (phenylpropanoids), i.e. the B and C rings of flavonoids (Tapas et a. 2008). Also, the metabolism of carbohydrates and fatty acids produces acetic acid. Activated acetic acid (acetyl -CoA) is converted into malonyl-CoA and this biosynthetic pathway represents the acetate-malonate cycle. Ring A of flavonoids is formed through this biosynthetic pathway.

Phenylalanine-ammonium lyase catalyzes the reaction of releasing ammonia from phenylalanine, which leads to the formation of cinnamic acid. By introducing a hydroxyl group in the para-position of cinnamic acid, coumarin acid is formed. Further hydroxylation of coumarin acid in position 3 leads to the formation of caffeine, while hydroxylation in positions 3 and 5 with simultaneous methylation of these positions leads to the formation of ferulic and sinapic acids.

As these compounds contain a phenyl group and a side chain of three C atoms, they are called phenylpropanoids (C6–C3) and serve as precursors in the synthesis of lignin and many other compounds (Marais et al. 2006; Casana l et al. 2013).

Flavonoids, including flavones, isoflavones and anthocyanidins, are formed by the condensation of phenylpropanoids. With the participation of three molecules of malonyl-coenzyme A, and then by condensation of the newly formed unit with *p*-coumarin acid, a chalcone is formed, which gradually cyclises and leads to the formation of naringenin (flavone). This point in biosynthesis represents a "branching point" and from here the synthesis of various structural classes of flavonoids takes place. The general path of synthesis (Figure 1) of phenylpropanoids and flavonoids takes place as follows:

- Acetyl-CoA: carbon dioxide ligase (ACC) that catalyzes the ATP-dependent carboxylation of acetyl-CoA, with Mg^{2+} as a cofactor, to malonyl-CoA.
- Phenylalanine-ammonium lyase (PAL) converts phenylalanine to *trans*-cinnamate (*E*-cinnamate), *by trans* -elimination of ammonia and pro-3 *S* protons.
- Cinnamate-4-hydroxylase (C4H) catalyzes the hydroxylation *of trans*-cinnamate to *trans*-4-coumarate.
- 4-Coumarate-CoA ligase (4CL) activates hydroxycinnamic acids to enter later parts of the phenylpropanoid biosynthetic pathway, forming the corresponding thiol ester of CoA. 4-Coumarate is the main one in the synthesis of flavonoids, but 4CL also accepts other hydroxycinnamic acids as substrates.
- Chalcone synthetase (CHS) catalyzes a series of sequential decarboxylation and condensation reactions, using 4-coumaroyl-CoA and three molecules of malonyl-CoA, with the aim of creating polyketide intermediates that undergo cyclization and aromatization reactions, which lead to the formation of the A-ring and the resulting chalcone structure.
- Chalcone isomerase (CHI) catalyzes the stereospecific isomerization of chalcone to its corresponding (2 *S*)-flavanone.
- Flavanone-3 β -hydroxylase (F3H) catalyzes the conversion of (2 *S*)-flavanones into the corresponding (2*R*, 3*R*)-dihydroflavonols.
- Dihydroflavonol-4-reductase (DFR) catalyzes the stereospecific conversion of (2*R*, 3*R*)-*trans*-dihydroflavonols to the corresponding (2*R*, 3*S*, 4*S*)-flavan-2,3-*trans*-3,4-*cis*-diol (leucoanthocyanidin).
- Anthocyanidin synthase (ANS) catalyzes the reduction of leucoanthocyanidins to the corresponding anthocyanidins (Casanal et al. 2013).

Variations in the structure of hydroxybenzoic acid derivatives (Figure 1) are caused by hydroxylation or methylation of the aromatic nucleus. In the plant world, *p*-hydroxybenzoic, vanillic, syringic and protocatechinic acids occur most often. Gallic acid participates in the formation of chirolyzing gallotannins, and its condensation produces dimerellagic acid. Ellagic acid is also part of ellagiotanin.

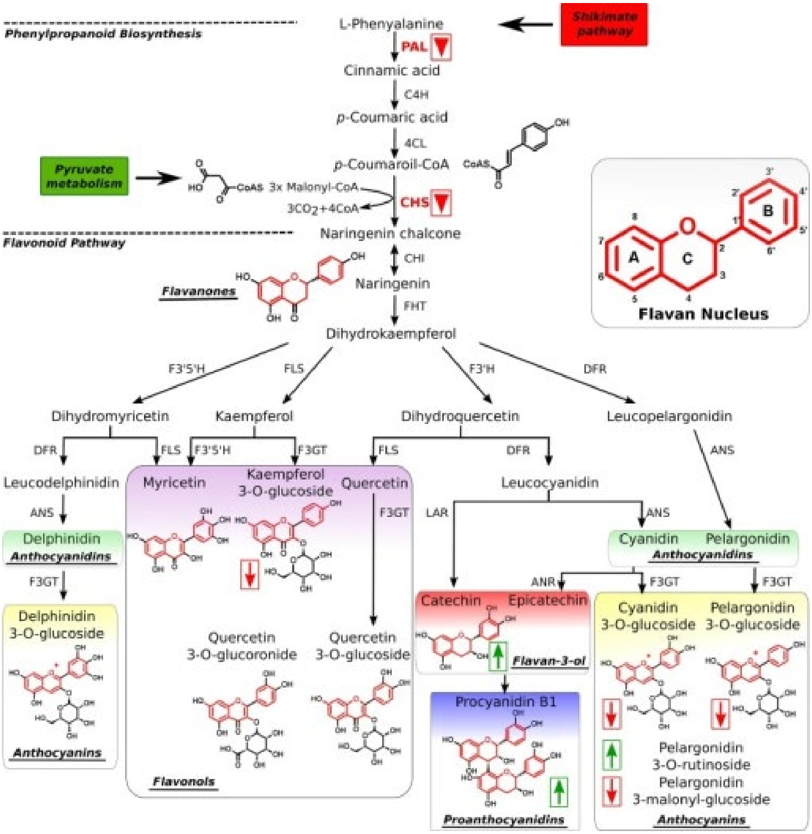


Figure 1. Scheme of biosynthesis of flavonoids (Casanal et al. 2013).

Hydroxycinnamic acid derivatives have a C6-C3 structure. The most common derivatives of hydroxycinnamic acid are *p*-coumaric, caffeic, ferulic and sinapic acid. Hydroxycinnamic acid derivatives are available in various conjugated forms. Conjugated forms are esters of hydroxy acids such as: quinic acid, shikimic acid and tartaric acid, as well as their glycosides.

Free forms indicate enzymatic hydrolysis in the plant tissue during extraction (Jiang and Gurdial, 1998; Jašić 2010).

Monophenols and phenolic acids take part in the hydrogen atom release reaction and free radical capture reactions. It has been confirmed that polyphenols are more effective antioxidants than monophenols, and that the introduction of another hydroxyl group in the ortho and para position significantly increases this activity. Thus, the antioxidant activity of ortho-diphenolic acids, protocatechinic and caffeic, is higher than the corresponding monophenolic acids of the same structure, *p*-hydroxybenzoic and *p*-coumaric acids. Gallic acid with three hydroxyl groups is a more effective antioxidant than protocatechinic acid. However, it was found that the presence of more than three hydroxyl groups on the aromatic backbone does not improve the antioxidant efficiency of the molecule. The results also shown that the presence of one or two methoxy groups in the ortho position increases the antioxidant activity of monophenols: sinapic acid is more active than ferulic acid, and ferulic acid is more active than *p*-coumaric acid; syringic acid is more effective than vanillic and *p* -hydroxybenzoic acids. Several authors have argued that the presence of an electron-donating (hydroxy or methoxy) group in the ortho-position significantly contributes to the stability of the formed aroxyl radicals. Ferulic and vanillic acid shown significantly weaker antioxidant activity than caffeine and protocatechinic acid (Harborne and Baxter 1999; Harborne et al. 2000).

Pratt and Birac (1979) concluded that the methoxy group in the ortho-position of ferulic acid stabilises the phenoxyl radical that is formed in the reaction with free radicals, thus increasing its antioxidant potential compared to *p*-coumaric acid. Derivatives of hydroxycinnamic acid are generally better antioxidants than derivatives of hydroxybenzoic acid due to the presence of a double bond that participates in the stabilization of the generated aroxyl radical by resonance. The similar antioxidant activity of gallic acid and propyl gallate indicates a weak effect of esterification on the antioxidant activity of phenolic acids. Pratt and Birac (1979) stated that the activity of caffeic acid is greater than ferulic and *p*-coumaric acid due to the presence of a second hydroxyl group and the formation of intramolecular hydrogen bonds.

The proton from the carboxyl group has little effect on the antioxidant activity. Thus, the activities of chlorogenic acid and caffeic acid during the inhibition of lipid peroxidation are similar. The proton of the carboxyl group of caffeic acid is substituted by quinic acid in the chlorogenic acid molecule. The allyl group, which is present in cinnamic acid derivatives, increases the antioxidant potential compared to benzoic acid derivatives. Pratt and Hudson (1990) reported that the protective effect of caffeic acid (3,4-dihydroxycinnamic acid) during fat oxidation was greater than that of protocatechinic acid (3,4-dihydroxybenzoic acid). An acceptable explanation is that the resonance of the allylic residue increases the stability of the phenoxyl radical. Synthetic antioxidants used in the food industry are structurally also phenolic compounds: BHA-butylated hydroxyanisole, BHT-butylated hydroxytoluene, TBHQ-2-*tert*-butylhydroxyquinone and PG-propyl gallate. Their antioxidant activity is closely related to their structure, especially their ability to release a hydrogen atom and stabilise the product by delocalizing electrons in the aromatic nucleus (Piao et al. 2010). Stilbenes have a basic molecular structure of C6-C2-C6 (Figure 2). The dominant compound in the stilbene group is resveratrol. Stilbenes are an effective defense against fungi in plants. The special importance of stilbenes is their ability to retain antifungal activity in the human body as well. They are also powerful antioxidants and, according to some studies, have an anti-cancer effect (Kumar and Abhay 2013).

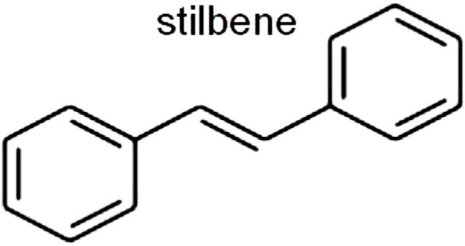
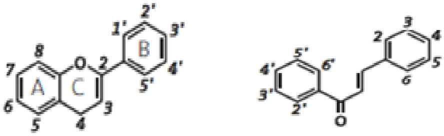
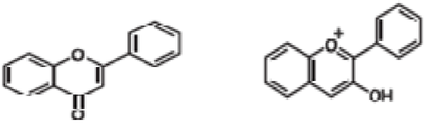


Figure 2. Basic chemical structure of stilbene

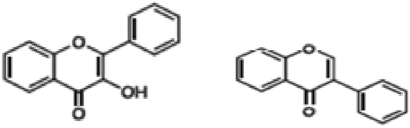
Stilbene glycosides: malberoside A, *cis* -mulberoside A and resveratrol-4,3’-di-O-β-glucopyranoside were found in the leaf and fruit of white mulberry. Two years later, the presence of five stilbene glycosides in the bark and root of mulberry (*M. alba*) was proven: malberoside A, *cis*-alberoside A, resveratrol-4,3’-di-O-β-glucopyranoside, oxyresveratrol-2-O-β-D-glucopyranoside and oxyresveratrol-3’-O-β-D-glucopyranoside. The presence of flavonoids and stilbene glycosides together with resveratrol and maclurin is associated with tyrosinase inhibition. Flavonoids as proton donors prevent the reduction of dehydroascorbic acid or inhibit certain enzymes (hyaluronidase, elastase and catechol-O-methyltransferase) and thus slow down the process of elastin decomposition. Genistein has been found to act as a phytoestrogen and prevent the formation of breast and prostate tumors (Cook and Samman 1996; Bertelli et al. 1998; Baumgertel et al. 2010). Flavonoids include compounds such as catechins, anthocyanins and various forms of flavonoids (Figure 3). In addition to having an antioxidant effect, they act antimicrobially, inhibit various enzymes, have a cytotoxic antitumor effect and act as estrogens. As components of food of plant origin, many of the various flavonoids known so far are part of a regular diet. The variety of flavonoids is controlled by the genes of the plant, but also by the maturity of the plant, the climate and the method of cultivation. In nature, flavonoids are found mainly in the form of glycosides, i.e. they are linked to different sugar molecules (Marais et al. 2006).



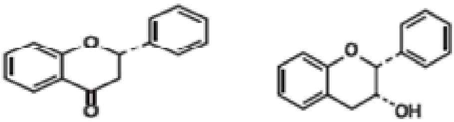
flavonoid chalcone



flavone anthocyanin



flavanol isoflavon



flavonon flavanol

Figure 3. Basic chemical structures of different subclasses of flavonoids

Rich sources of flavonoids are: fruits and vegetables, green and black tea, chocolate, red wine and berries. They are found in plants and are related to the process of photosynthesis, so they are present in fruits, vegetables, seeds, flowers, but also in tea, wine, honey and propolis (Harborne and Baxter 1999; Bertoncelj 2008). Flavonols and, to a lesser extent, flavones were found in almost every plant. In plants, free flavonoids are aglycones (e.g. without an attached saccharide) that occur in a variety of structural forms. Only a certain number of flavonoids give colour to plants due to absorption in the visible part of the spectrum. With an increase in the number of hydroxyl groups, the antioxidant activity of flavonoids increases (Kazazić 2004; Andersen et al. 2005). Flavanones and flavones are often found together, e.g. in southern fruits and are associated with specific enzymes unlike flavones and flavonols which are mutually exclusive in many plants (Brand 2010).

Flavonoids in plants are mainly in the form of 3-O-glycosides or polymers. Polymerization of flavanols into tannins and other complex compounds occurs, for example, during fermentation by enzymatic oxidation of green leaves into black tea. Tannins or gallotannins are esters of 3,4,5-trihydroxybenzoic acid. The galloyl group of those tannins and monomeric catechins in green tea is partially responsible for the chelating and free radical scavenging properties. Condensed tannins or proanthocyanidins consist of flavanol units of which there can be up to 17 in one molecule. For human consumption, the most important of these compounds are procyanidins, which consist of (+)-catechin and (–)-epicatein monomers. (–)-Catechins and (+)-epicatechins are phytotoxic and some plants synthesise them in their roots to prevent other plants from colonizing that territory (Brand 2010). In the plants themselves, flavonoids act as antioxidants, antimicrobials, as photoreceptors and as agents for attracting attention, repelling food and protecting against UV radiation. Obviously, these are compounds that have an important role in maintaining and protecting the vital functions of plants, and having entered them through food, they have a similar role for other living beings. Because of all the positive properties, these compounds are the subject of research by many scientists (Macheix et al. 1990; Soler et al. 1995; Anklam 1998; Alvarez-Suarez et al. 2009).

The antioxidant activity of these compounds is primarily the result of their ability to be donors of hydrogen atoms and to remove free radicals as such, with the formation of the phenoxyl radical, which is stabilised by resonance and becomes less reactive. Increasing the stability of the resulting phenoxy radical increases the possibility of recombinant reactions that lead to the cessation of free radical processes. The phenoxy radical formed during the "capture" of the radical by a phenolic compound is capable of oxidizing both proteins and lipids. As a consequence, the role of biological antioxidants can be limited by their ability to cause cytotoxic and genotoxic effects, so the reactivity of phenoxy radicals must be considered as a critical point in the development of new antioxidant preparations (Cuvelier et al. 1992; Dai et al. 2010; Herken et al. 2010). The protective role of flavonoids in biological systems is attributed to their ability to pair ("capture") free radical electrons, chelate metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+}), activate antioxidant enzymes and inhibit oxidases. The mechanism of action of flavonoids at the molecular level in biological systems has not been fully elucidated, both because of the great difference in chemical properties and because of their great structural heterogeneity. These substances can be found in free form or more often in the form of glycosides or in the form of complexes with organic acids, amines, lipids, carbohydrates and other polyphenolic compounds. The biological activity of flavonoids is solely due to the aglycone part of the molecule (Pokorny 1987; Mei et al. 2006).

Phenolic compounds in plants are not evenly distributed at the tissue, cellular, and subcellular levels. Insoluble phenols are an integral part of the cell wall, while soluble phenols are found inside cell vacuoles. At the tissue level, the superficial layers contain higher levels of phenols than those found in their central parts. Cell wall phenolics, such as lignins and hydroxycinnamic acid, are associated with various cellular components. These compounds contribute to the mechanical resistance of the cell wall, have a regulatory role in the growth and morphogenesis of the plant, as well as in the reaction to stress and pathogens (Shahidi and Nack 1995; Mishan 2009).

The accumulation of polyphenolic compounds also varies depending on the physiological state of the plant, as a result of the balance between biosynthesis and further metabolism. Numerous studies confirm that the concentration of polyphenolic compounds is lower in ripe fruit, except for red fruits where flavonoids and anthocyanins accumulate at the end of ripening. In food, they appear as glycosides or esters with other compounds such as: sterols, alcohols, glucosides and fatty acids (Harborne and Baxter 1999; Grbović 2010). They have strong antioxidant, anti-inflammatory, anti-cancer and antimicrobial activity, and have also shown a positive effect in the treatment of many cardiovascular diseases and prevention of atherosclerosis (Chang et al. 2001; Gokče et al. 2008).

Dai and Mumper (2010) shown that phenolic acids show a stronger antioxidant effect than vitamins C and E, carotenoids and many commercial synthetic preparations. The metabolic pathway of flavonoid biosynthesis is probably the best studied of all plant secondary metabolites. It represents one part of a long chain of phenylpropanoid metabolism, which produces a large number of secondary metabolites such as: phenolic acids, lignin, lignans and stilbenes. The key precursor for their synthesis is phenylalanine, which is produced in the so-called Shikimata process (Jiang and Singh 1998; Vargas and Randy 2010). Flavonoids are bioenzymatically synthesised in cytosols and their further transport takes place through various cellular processes. They are involved in pigmentation and are located in vacuoles. Anthocyanins and proanthocyanidins are mostly coloured and most flavones, flavonols and isoflavones are colourless. The biosynthesis of flavonoids is an enzymatic process, but the genetic structure of the plant plays a crucial role, so various models are being developed in biotechnology with the aim of studying the formation of bioflavonoids (Marais et al. 2006). The complexity of flavonoid biosynthesis is shown in the diagram (Figure 4).

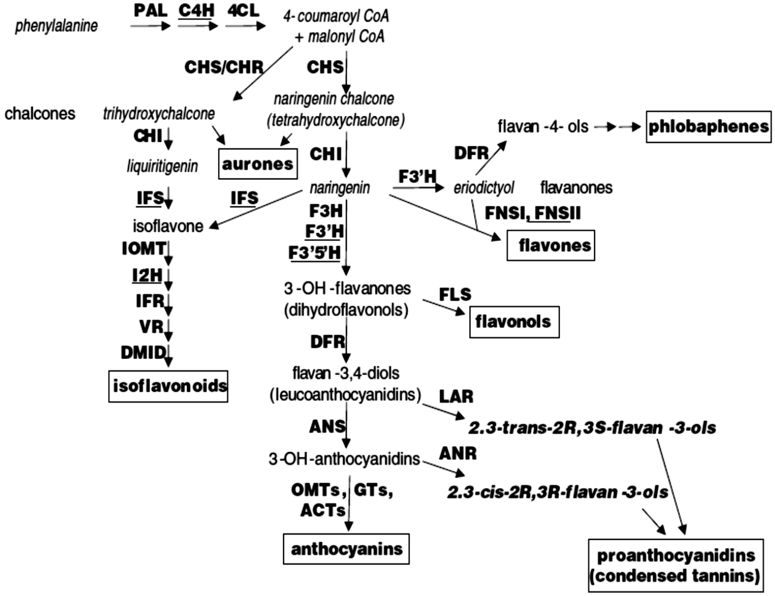


Figure 4. Schematic representation of the enzymatic biosynthesis of flavonols, anthocyanins, proanthocyanidins, phlobaphen, aurones, flavones and isoflavones (Marais et al. 2006).

The metabolism of the formation of flavonoids is a very complex process that is determined by the genetic and enzymatic structure of the plant. As many studies show, each group of flavonoids plays a specific role in plants, both pigmented and non-pigmented flavonoids (flavonols, flavones and isoflavones) (Harborne and Baxter 1999; Alvarez-Suarez et al. 2009).

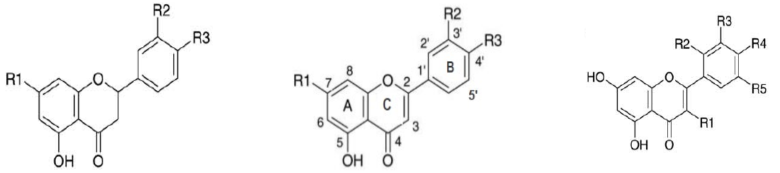
Chemical structure of flavonoids

Phenolic compounds as a widespread heterogeneous group of secondary plant metabolites are substances that have one or more aromatic rings with one or more hydroxyl groups in their structure (Harborne and Baxter 1999). Considering the great diversity of plant phenols (Table 2), their classification is very complex, so different classifications are often found in the literature, mainly according to chemical structure and biosynthetic origin (Harborne and Baxter 1999; Kazazić 2004; Bertoncelj 2008).

The word polyphenol is derived from the ancient *Greek* word *poly* which means “many” and the word phenol which comes from the phenyl ($-C_6H_5$) group connected to the hydroxyl (-OH) group. Polyphenols include more than 8 thousand compounds different chemical structures, from simple hydroxymethyl acids and anthocyanins (plant pigments) to more complex flavonoids and tannins whose basic feature is the presence of one or more hydroxylated benzene rings. They have a molecular weight of 500 to 4000 Da (g/mol). They are built from 12 to 16 phenolic groups with five to seven aromatic rings (Marais et al. 2006).

Flavonoids or bioflavonoids are non-ketone polyhydroxy polyphenolic compounds. They are mostly found in the skins of fruits and vegetables. About 4 to 7 thousand different types of flavonoids are known (Cushnie et al. 2005). Flavonoids (*Latin flavus* means yellow) are a group of polyphenolic compounds found in many plants, concentrated in seeds, skin or skin of fruits, bark of trees, leaves and flowers, that is, in the plant kingdom (Ferreira et al. 2009; Lachman et al. 2010).

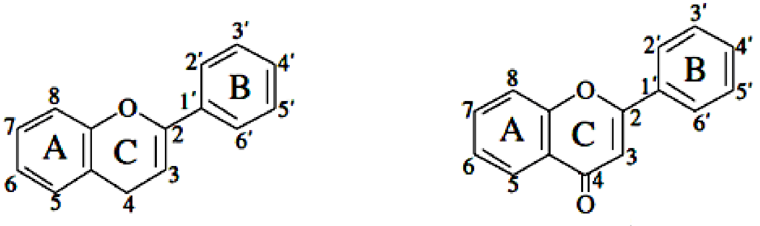
Common structural formulas of flavonoids are given in Figure 5 (a – flavanones, b – flavones, c – flavonols). In general, the term flavonoid is used to describe a group of natural compounds that have a C6 – C3 – C6 bond in their structure, that is, a phenylbenzopyran structure. In relation to the position of the aromatic ring on the benzopyran cycle, natural flavonoids are divided into three groups: flavonoids (2 – benzopyrans), isoflavonoids (3 – benzopyrans) and neoflavonoids (4 – benzopyrans). Flavonoids are divided into several subgroups: chalcones, flavans, flavones, flavonols, isoflavones, flavanones, flavanonols and anthocyanins, whose basic structural formulas are given in Figure 6. In addition to sugar, the substitution groups found on the basic structure are hydroxyl and methoxy groups, which contributes to the great variety and large number of these compounds (Marais et al. 2006; Brand 2010).



(a) (b) (c)

Figure 5. Chemical structure of flavanones (a), flavones (b) and flavonols (c)

The basic flavonoid structure consists of three phenolic rings (A, B and C rings), in which all 15 carbon atoms are interconnected. The benzene ring A is condensed with a three-membered aliphatic chain that, together with oxygen, forms a six-membered ring C, and the benzene ring B is located at position 2 of ring C. For flavonoids that have a carbonyl group attached to the C-4 atom of ring C, they term 4-oxo-flavonoids. The diversity of the flavonoid class is based on the oxidation of the C-ring (Marinova et al. 1992; Marais et al. 2006).



(a) (b)

Figure 6. Basic structure of flavanoids: (a)-flavan core, (b)-oxo-flavonoid core.

Differences between individual flavonoid subgroups arise from variations in the number and arrangement of hydroxyl groups, as well as from the nature and degree of their alkylation and/or glycosidation. The most common are flavones and flavonols with hydroxyl groups in the 3’- and 4’- positions in ring B, and less frequently those with a hydroxyl group only in the 4’- position (Figure 6). Glycosidation with flavonoids occurs most often in the 3- position, and less often in the 7- position. The most common sugar is glucose, but galactose, rhamnose and xylose also occur. Glycosidated cyanidins are called cyanins. Flavonoids present in food differ in the position of hydroxyl, methoxy and glycosidic groups and in the conjugation between rings A and B (McKibben and Nicki 2002; Kumar and Pandey 2013).

Phenolic compounds form one of the main group secondary metabolites of plant life that have different chemical structures and functions, but in generaly, its aromatic ring wears one or more of hydroxy substituents. Phenolic flavonoids have diphenylpropane (C_6-C_3 -C_6) skeleton, such as monomeric compounds of flavanols, flavanones, anthocyanidins, flavones and flavonone. Flavonoids also include folic acid (Cuvelier et al. 1992; Bertelli et al. 1998; Arsenijević 2001).

The term phenolic acid includes all compounds that contain at least one phenolic and one carboxylic group, and in practice it usually includes two groups of compounds: a) benzoic acid derivatives and b) cinnamic acid derivatives (Figure 7).

Table 1. Overview of structural classes of phenols

Ordinal number	Number of C atoms	C - skeleton	Classes of plant phenols
1.	6	C6	Simple phenols
2.	7	C6-C1	Hydroxybenzoates
3.	8	C6-C2	Acetophenones and phenylacetates
4.	9	C6-C3	Hydroxycinnamates, phenylpropenes
5.	10	C6-C4	Coumarins and chromones
6.	13	C6-C1-C6	Naphthoquinones
7.	14	C6-C2-C6	Xanthones
8.	15	C6-C3-C6	Stilbenes and anthraquinones
9.	18	(C6-C3)2	Flavonoids
10.	30	(C6-C3-C6)2	Lignans
11.	n	(C6) n	Bioflavonoids and catenol melanins
12.	n	(C6-C3) n	Lignins
13.	n	(C6-C3-C6)n	Condensed tannins

These compounds are formed by the condensation of shikimic acid with phosphoenolpyruvate to give chorismic acid. Additional reactions of decarboxylation, transamination and deamination produce cinnamic (3-phenylpropene) acid and hydroxy cinnamic acids. Subsequent reactions of hydroxylation, methoxylation, etc. derivatives of cinnamic acid are formed:

- p-coumaric acid (p-hydroxy cinnamic acid);
- caffeic acid (2,3-dihydroxy cinnamic acid);
- ferulic acid (2-methoxy-3-hydroxy cinnamic acid);
- sinapic acid (2,4-dimethoxy-3-hydroxy cinnamic acid).

More and more attention is paid to the study of the chemical structure and pharmacological action of phenolic acids and their glycosides. It has been established that many drugs that are traditionally used contain exactly these compounds as active ingredients (Prior and Guohua 2000; Pokorny et al. 2001).

Numerous studies indicate that cinnamic acid derivatives (C6-C3) are more effective antioxidants compared to substituted benzoic acid (C6-C1), most likely due to the formation of a free radical intermediate, the stability of which is increased due to the conjugation of the aromatic ring with a propens side chain (Nijveldt et al. 2001).

The antioxidant potential of benzoic and cinnamic acid derivatives varies significantly depending on the number of hydroxyl groups of the benzene ring, and is proportional to the number of electron-donating substituents, as well as the degree of stabilization (number of resonance structures) of the resulting free radical intermediates. Another important group of polyphenols are anthocyanins (Figure 8).

Anthocyanins (*Greek: anthos* – flowers, *kyanos* – blue*)* belong to the group of flavonoids. They are water-soluble plant pigments that give flowers, fruits and vegetables their blue, purple and red colours. Anthocyanins are anthocyanidin glycosides with the characteristic chemical structure of flavonoids C_6-C_3-C_6.

There are six basic anthocyanidins: cyanidin, delphinidin, pelargonidin, peonidin, petunidin and malvidin, and by binding sugar to these basic anthocyanidins, anthocyanin molecules are formed. The different chemical structures of anthocyanins show a different colour depending on the pH of the solution in which they are found (Vargas et al. 2003; Kazazić 2004; Dixon et al. 2005).