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Isoflavonoids in food

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Declaration

I, Martina Karlíková, hereby declare that I have written the following thesis entitled "Isoflavonoids in food" independently and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague, 2018 ....

Martina Karlíková

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Abstract

Isoflavonoids are secondary metabolites of plants with a wide range of biological effects. Among others effects, they act as phytoestrogens with activity of endogenous estrogens that can show both, positive and negative effects. The most important for humans is the alleviation of menopausal symptoms and for plants they play a role in the defence against pathogens and supporting the rhizobiological symbiosis. The aim of this study was to create an overview of foods and beverages containing isoflavonoids. Most isoflavonoids were found in plants from the *Fabaceae* family, where the main representative is soy (*Glycine max*), which contains major isoflavonoids such as daidzein, genistein and formononetine in smaller dose. Another important representatives are cowpea (*Vigna unguiculata*), alfalfa (*Medicago sativa*) and red clover (*Trifolium* sp.), where biochinin A can be also found. However, there is also other 59 plant families where isoflavonoids have been found, for example in iris (*Iris potanini*). Isoflavones, as the main structure of isoflavonoids, exhibit estrogenic, antifungal and anti-cancerogenic properties. The amount of their consumption is also important. It has been found that in the Asian countries, where isoflavonoids are eaten more in fermented form, positive effects outweigh the negative ones, in opposite with Western diet.

Key words:

phytoestrogens, flavonoids, isoflavones, *Leguminoseae*, *Fabaceae*, soy, dietary habits, daidzein, genistein, menopausal symptoms

Abstrakt

Isoflavonoidy jsou sekundární metabolity rostlin s širokým spektrem biologických účinků. Kromě jiných působí jako fytoestrogeny, které mohou jakožto endogenní estrogeny vykazovat pozitivní i negativní efekty. Nejdůležitějším významem pro člověka je zmírnění menopausálních příznaků a pro rostliny hrají roli v obraně před patogeny a v navozování rhizobiální symbiózy. Cílem této studie bylo vytvořit přehled poravin a nápojů s obsahem isoflavonoidů. Nejvíce isoflavonoidů bylo nalezeno u rostlin z čeledi bobovitých (*Fabaceae*), kde hlavním zástupcem je sója (*Glycine max*), která obsahuje hlavní isoflavonoidy jako daidzein, genistein a v menší dávce i formononetin. Dalším zástupcem je vigna (*Vigna unguiculata*), tolice vojtěška (*Medicago sativa*) nebo jetel červený (*Trifolium* sp.), kde se může objevovat i biochanin A. Nalezeno bylo ale i dalších 59 rostlinných čeledí, kde jako příklad můžeme uvést iris (*Iris potanini*). Isoflavony jakožto hlavní struktura isoflavonoidů tedy vykazují estrogenní, protiplísňové a protirakovinotvorvé vlastnosti. Důležité je i množství jejich konzumace. Bylo zjištěno, že u asijské populace, která isoflavonoidy pojídá více ve fermentované formě, pozitivní efekty převažují nad negativními oproti stravě západních zemí.

Klíčová slova:

fytoestrogeny, flavonoidy, isoflavony, *Leguminoseae*, *Fabaceae*, sója, stravovací návyky, daidzein, genistein, menopauzální příznaky

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1. Introduction

World of plants is very diverse. Plants synthesize many compounds that can be divided into primary and secondary metabolites. Isoflavonoids belong to secondary ones and until these days about 1,600 isoflavonoids have been identified (Veitch 2007). These secondary metabolites are recently subject of many studies. Isoflavonoids are formed from a class of flavonoids which belong to important antioxidants synthesized only in plants. As polyfenolic substances are isoflavonoids compose of 15 carbon structures with two benzene rings. The chemical structure of isoflavonoids are similar to female sex hormone - estrogen. This similarity in structure allow them to interfere with the action of estrogen. It is why isoflavonoids are often called "phytoestrogens". Phytoestrogens are non-steroidal secondary compounds with similar structure and biological activity as human hormone estrogen. When the hormone level of estrogen is high, isoflavonoids can also reduce the effect of the estrogen on mammalian cells and skin layers (Bradbury & White 1954). Due to their ability to bind on estrogen receptor, it may work as potential prevention for many diseases including osteoporosis, cardiovascular diseases, breast, prostate and colon cancer and menopausal symptoms (Corwell et al. 2004). Other biological role is to protect plants from pathogens and act as part of a plant's defence mechanism. But more important for plants is positive effect on rhizobial symbiosis. Isoflavonoids produced in roots induce binding free oxygen from air and give it to the plants. They induce symbioses between plants and diazotrofic bacteria from rhizobia (Pičmanová 2008).

Isoflavonoids are from the phenylpropanoid pathway synthesized predominantly in leguminous plants family *Fabaceae*. Simple isoflavonoids are dietary phytoestrogens and their glycosides. Great number of them has been identified in the form of non-active glycosides (daidzein and genistein) and in the form of 4'-methylated derivatives (formononetin and biochanin A). In general, isoflavonoid compounds are primarily found in legumes, but they were also found in a considerable number of non-legumes. Recently, about 59 non-leguminous families also occur isoflavonoids (Veitch 2007; Lapčík 2007). Soy (*Glycine max*) as legumes containing 50-300 mg of genistein, daidzeine and their glycosides per 100 g of net weight (Lapčík 2004) in comparison with non-legumes iris (*Iris* sp.) with less amount of 5-140 μg per 100 g dry weight (Lapčík 2007).

Risk associated with consumption of isoflavonoid is reflected on sheep or cows conditions. When content of isoflavonoids in fodder is high it may change their ability to be fertile (Vrzáňová & Heresová 2003). The risk is also associated with their milk, which is farm product and further is sold to human where can cause further problems.

Legumes and their content of isoflavonoids played an important role in the diets differences between Asian and Western countries. The most commonly consumed legume is the soybean, but only 30 % of western population have ability to metabolize them (Moon et al. 2006). Consuming of isoflavonoids can influence symptoms of menopause, hormone tumors and other diseases. In some studies were proved that Japan people are under a lesser health risk than people with western lifestyle where isoflavonoids consumption is mainly in unfermented soy and soy products (Okabe et al. 2011).

2. Objectives and Methodology

The objective of this thesis was to collect and summarize which food or drinks content isoflavonoids and find their abundance in leguminous and non-leguminous family. Another point of this work was find if the phytoestrogenic activity of isoflavonoids affect plants or human body. The aim was also to evaluate how the diet-structure and geographical area affect people from Asia and Western countries and find some different signs between high or low consumption of isoflavonoids.

This study is a form of literature review. Data were collected from main scientific web databases Web of Knowledge, Scopus, Science Direct, etc., specialized books and scientific journals mostly with the use of keywords “isoflavonoids”, “phytoestrogens” and “isoflavones”. All sources are listed in the references.

3. Literature Review

Isoflavonoids are secondary metabolites of plants with wide range of biological effects. They belong to the most studied group of polyphenols - flavonoids. Difference between 15 carbon structure of flavonoids and isoflavonoids is in different position of ring B benzene molecule (Pandey et al. 2014). For their function as endocrine estrogens they are called as "phytoestrogens". These phytoestrogens have positive effects on human health, including the prevention of cancer and menopausal symptoms. However, there are still the potential risks associated with their consumption. Special importance has isoflavonoids for plants themselves, especially in the defence against pathogens thanks to phytoalexins or phytoanticipins and in right function of rhizobial symbiosis (Pičmanová 2008). Most isoflavonoids are produced by the plants family *Leguminoseae* family – *Fabaceae*, but there are also known few others plant families. In connection with the text above is important to mention also doses of isoflavonoids and consumption area. It is known that people from Asia consume more isoflavonoids (in soy products) and they have less symptoms of certain health disorders in comparison with western countries (Setchell et al. 2002).

3.1. Polyphenolic substances

Polyphenols are a group of chemical compounds contained in plants. They are characterized by the presence of more than one phenolic unit or building block in the molecule. Polyphenols are generally divided into hydrolysable tannins (esters of gallic acid and glucose or other sugars) and phenylpropanoids, for example lignans, flavonoids and condensed tannins. The most well-studied polyphenols are flavonoids among which thousands of compounds, including flavonols, flavones, catechins (flavan-3-ols), flavanones, anthocyanidins and isoflavonoids **see Fig.1** (Reynaud et al. 2005).

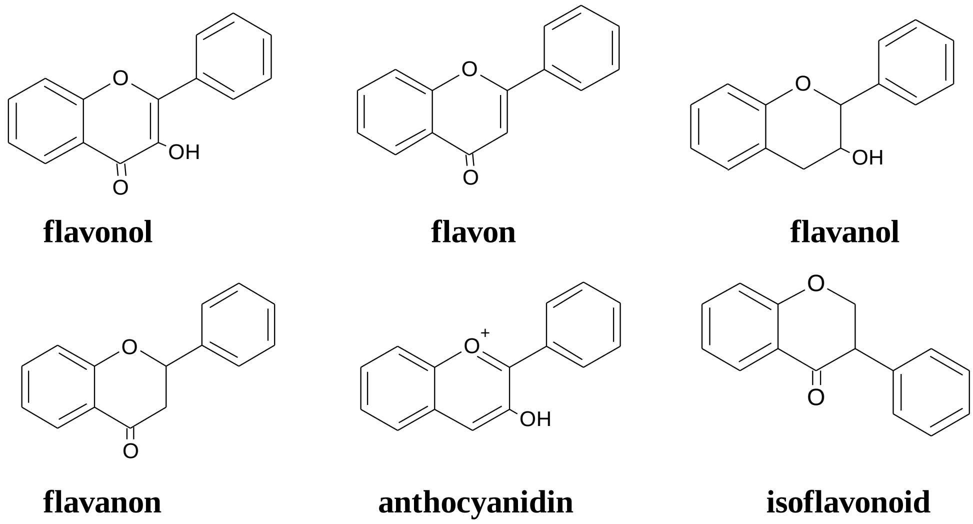


Figure 1: The skeletons of the flavonoids with three-ring structures

Flavonoids are among polyphenolic compounds synthesized only in plants. They occur in higher plants, mainly in flowers, leaves and bark. We find them very abundantly in fruits and vegetables (especially in peel), grains and nuts (van Acker 2001). Flavonols are found in commonly consumed fruits, vegetables and other plant products such as citrus fruits, onions, papaya, broccoli, grapes, leafy vegetables, tea, chocolate, soy and cereals (Ronnie 2003). A major source of flavonoids are also beverages containing plant extracts, such as fruit juice, tea, wine and beer (van Acker 2001).

Flavonoids belong to the most important antioxidants. They prevent lipid peroxidation, eliminate free oxygen radicals, bind and inactivate certain prooxidation ions of metals (iron, copper) (Ronnie 2003). Their action is the basis of prevention of diseases related to oxidative damage of membranes, proteins and DNA (Ferguson 2001). These include cardiovascular disease, cancer and inflammation. It appears that during the therapy and prevention of these diseases, consumption of flavonoid-containing foods is more appropriate than antioxidants such as Vitamin C and E (Ronnie 2003). Flavonoids have been designed to be used as a chemopreventive supplement for heart and liver diseases as well as for cancer diseases. The antioxidant action of flavonoids is beneficial even in cataracts and macular degradation (Sanderson et al. 1999).

Plants are affected by flavonoids in most of interactions and are also water-soluble dyes giving to flowers, fruits, and less often to the leaves, their colour. They act as a magnet for pollinators and for seed transporters. The location of flavonoids in plant tissue is dependent on the species of plant and, in addition, they occur partially loosely and partly in the form of glycosides. Soluble glycosides are mainly found in vacuoles. In the flowers, the flavonoid carriers are epidermal cells. Flavonoids are also in the fetus, seeds, and even in pollen grains. Flavonols and flavons are yellow compounds and important food colouring (flavus - yellow). Flavanols and flavanons are colourless or light-yellow compounds, and as colourants are irrelevant. Flavonoids are chemically related to anthocyanidins, respectively their glycosides anthocyanins. These are the widely-used pigments contained in all plant materials and make these plants more attractive due to orange, red, purple and blue pigments, depending on pH. Catechins and leucoanthocyanidins are colourless compounds and are subject to enzyme browning food. The chalcones form the yellow colour of the plant flowers (for example the dahlias) along with gold-yellow aurons. As the latter in the group of flavonoids are isoflavonoids, which form yellow pigments in soy, but as food colours are insignificant (Velíšek & Hajšlová 2009; van Acker 2001).

3.2. Phytoestrogens

The word phytoestrogen comes from Greek where "phyto" means a plant and "estrogen" is a hormone responsible for the mammalian females fertility.

Phytoestrogens can be divided into four main classes: isoflavonoids, flavonoids, lignans and coumestrol **see Fig. 2 & 3**. The most common of phytoestrogens are substances called isoflavonoids (often called soy isoflavonoids). They are found mainly in soybean and red clover.

Isoflavonoids are classified as both phytoestrogens and selective estrogen receptor modulators which belong to a group of endocrines estrogens. It means similar effects as sex steroid hormones – estrogens, but they are not steroidal compounds (Salgado & Donado-Pestana 2011). They are able to behave like estrogen but act as an estrogen antagonist (a substance that has the opposite effect to the body than the biological hormone estrogen, which the body synthesizes). They act on the body by attaching themselves to estrogen receptors - that is, in a nutshell, a sort of lock that unlocks the path to the cells and triggers various processes, and when the estrogen, which acts as a key, is triggered, the processes in the body start. Their effect lies in the fact that they can in the complex with the receptor bind to nuclear DNA and then affect transcription (Bradbury & White 1954).

The chemical structure of phytoestrogens differs from estrogen in the only common feature in the phenolic nucleus of the molecule that allows binding to the estrogen receptor and consequent metabolic activity and secretion similar to endogenous estrogen (Zand et al. 1998).

Our body not formed them, but we accept them in the foods we eat. So they are not essential substances (substances that the body cannot synthesize and must eat in the diet) and we cannot considered them as nutrients.

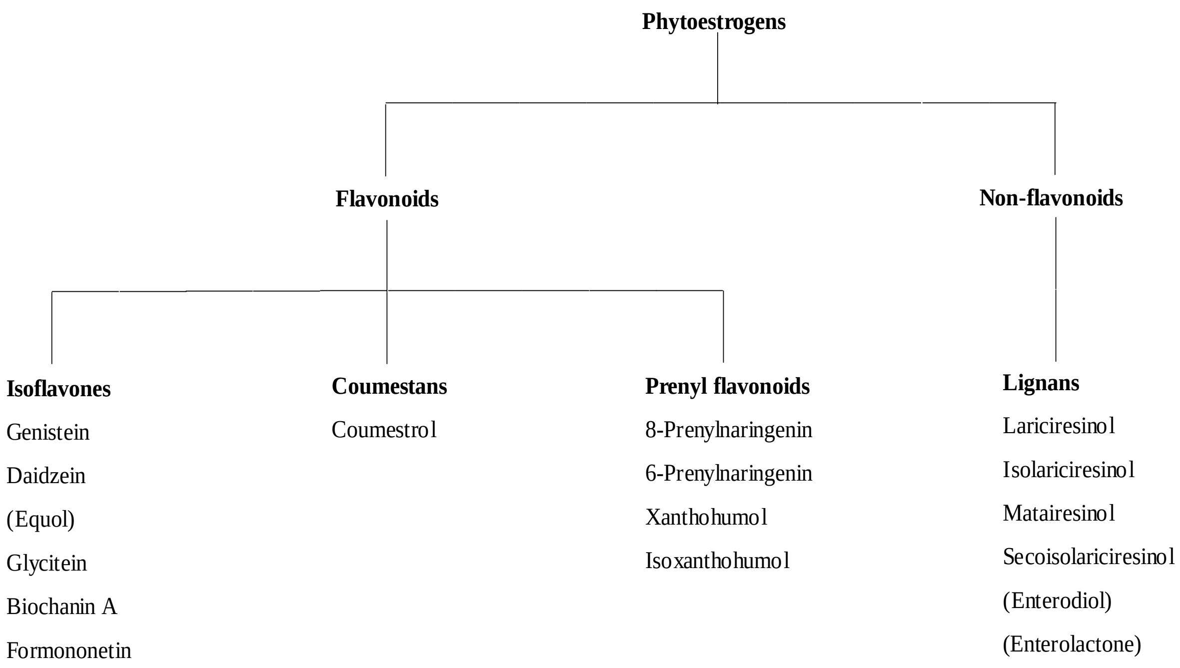


Figure 2: Phytoestrogens

(https://web.vscht.cz/~schulzov/Nutraceutika%20a%20FP/Fytoestrogeny2014.ppt)

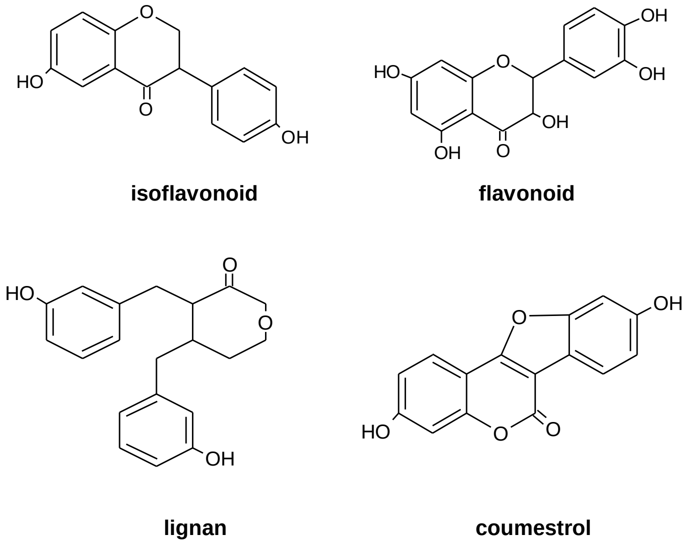


Figure 3: Four main classes of phytoestrogens

Phytoestrogens consist of more than 20 compounds and could be found in over 300 plants. They reduce the risk of osteoporosis, heart disease, breast cancer and menopausal symptoms. The topic of phytoestrogens deals with a wealth of literature and has been shown to have a positive effect on bone metabolism, fat and cholesterol metabolism, reduction of cardiovascular disease, osteoporosis and hormone-dependent cancers, mainly breast, uterus, prostate and colon (Ososki & Kennelly 2003).

Although the amount and concentration of phytoestrogens are different, vegetables have a high content of phytoestrogens, such as flaxseed, which is a rich source of lignans; soybeans and chickpeas have high concentrations of isoflavones. Foods containing isoflavone are highly consumed. Lignans can be also found in cereals, vegetables, and fruits (Kuhnle et al. 2009).

3.3. Isoflavonoids

Isoflavonoids are a group of low molecular weight secondary metabolites produced mainly from plants of the legume family (*Fabaceae,* syn. *Leguminosae*). 95 % aglycones of isoflavonoid structures known until 1988 have been described in the legume family and, as a result, isoflavonoids have long served as chemotaxonomic markers for this family (Reynaud et al. 2005).

Over the past twenty years, a number of new isoflavonoid structures have been identified thanks to considerable progress in analytical methods. Whereas in 1962 only 26 different isomers of isoflavonoids were described, in 1988 their number increased to 630 and in 1993 to 870 different isoflavonoids (Reynaud et al. 2005); the number of newly discovered isoflavones and the families in which they are occurring is still growing. At present, a total of about 1600 different isoflavonoids (including glycosides) are known, of which at least 225, primarily isoflavones, have been described in 59 non-leguminous families (Veitch 2007; Lapčík 2007).

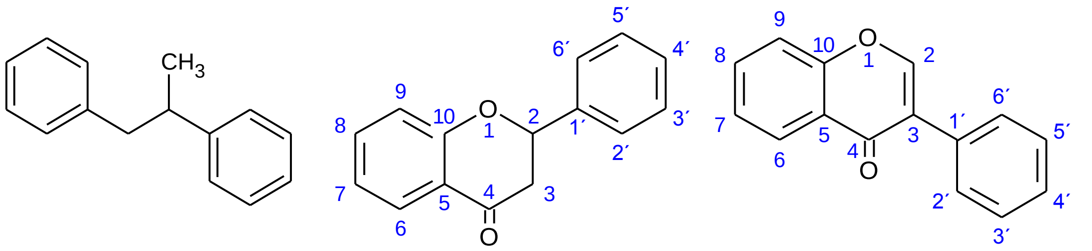
Isoflavonoid (a subgroup of flavonoids) are known to be highly potent antioxidants which have many health benefits, including protection against breast cancer, prostate cancer, menopausal symptoms, heart disease and osteoporosis. Isoflavones are produced by a branch of the general phenylpropanoid pathway biosynthesis that produces flavonoid compounds in legumes and stored as glucosyl- and malonyl-glucose conjugates (Graham 1991). Their occurrence is regrettably limited because isoflavone synthase, the enzyme required to convert their flavanone precursors, is unique only for legumes and a few other species (Rolfe 1988; Yu et al. 2000).

Due to a wide variety of biological effects, isoflavonoids have caused extraordinary interest in plant physiologists and pharmacologists in the last twenty years. Isoflavonoids are used as phytoalexins, phytoanticipins, insecticides and chemoattractants when introducing rhizobial symbiosis. Considerable attention also deserves due to their phytoestrogenic activity and as a healthy part of the human diet with antiviral, antioxidant and cancero-protective action. (Pičmanová 2008)

Besides the main isoflavonoid producers - legumes - a number of non-leguminous producers are also known to be used mainly in traditional medicine. A typical example is the iris (*Iris potanini*), which has been used in traditional mongolian healing since time immemorial to treat bacterial infections, inflammations and even cancer. Licorice root (*Glycyrrhiza echinata*) is commonly used in Chinese traditional medicine for treating inflammation, allergies, and asthma (Bielenberg 2001). And other non-leguminous species as kudzu (*Pueraria lobata*) known for its high content of isoflavonoids is used in traditional medicine in different parts of the world to treat jaundice, venereal diseases and kidney and hepatic stone removal (Reynaud et al. 2005).

3.3.1. Chemistry of isoflavonoids

Isoflavonoids are a large subclass of the most common plant polyphenols containing 15 carbon atoms, known as flavonoids (Crozier et al. 2009). Flavonoids are biological products contents 5000 different structures. Fifteen-carbon skelet of isoflavonoids (C6-C3-C6) is formally derived from 1,2-diphenylpropane **see Fig.4** (Reynaud et al. 2005). In isoflavonoids (3-phenylchroman), phenyl ring B is attached to the 3-position heterocyclic ring C as opposed to the 2-position linkage of flavonoids see Fig.4 (Pandey et al. 2014; Han et al. 2009).

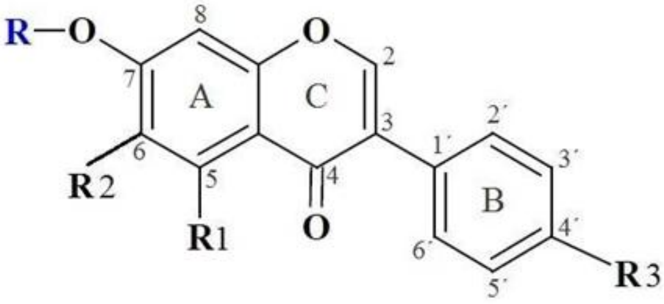


a) 1,2 difenylpropan **b)** flavon(2-fenylchromen-4-on) **c)**isoflavone (3-fenylchromen-4-on)

Figure 4: Structure of diphenylpropane skeleton, flavon and isoflavone

The flavonoid skeleton may carry various combinations of substituents **see Fig.5 & 6**, most typically hydroxyl-OH, methoxyl -OCH3 and also O- and C-glycosyls, according to which they are then divided into the groups I mentioned in chapter 3.1. Recently the most studied compounds are those with estrogenic activity. It contents isoflavonoids: daidzein (7,4´- dihydroxyisoflavone), genistein (5,7,4´-trihydroxyisoflavone), formononetin (7-hydroxy- 4´- methoxyisoflavone), glycitein (7,4´-dihydroxy-6-methoxyisoflavone) a biochanin A, (5,7- dihydroxy-4´-methoxyisoflavone) (Velíšek & Hajšlová 2009).

In the plant, isoflavonoids occur in the form of aglycons and glycosides (Reynaud et al. 2005). Generally, all glycosides have an intact component (aglycon) and a sugar component (glycosyl - the monovalent β-D-glucose residue). In this case, the aglycon is isoflavone and the sugar component - glycosyl – is monosaccharide β-D-glucose. These compounds are most commonly found in the form of glucosides and esters thereof with malonic acid. From the β-D-glucose monosaccharide, the glycosides are called glucosides (Velíšek & Hajšlová 2009). These biological compounds occur predominantly as 7-β-D-glucosides. The names of the glucosides of the individual isoflavones are daidzin from daidzein, genistein from genistein, ononin from formononetin and glycitin from glycitein (Velíšek & Cejpek 2008).



R = H	**R1**	**R2**	**R3**
daidzein	H	H	OH
genistein	OH	H	OH
formononetin	H	H	OCH_3
glycitein	H	OCH_3	OH
biochanin A	OH	H	OCH_3

Figure 5: Overview of significant isoflavonoids with general structure (Velíšek & Hajšlová 2009)

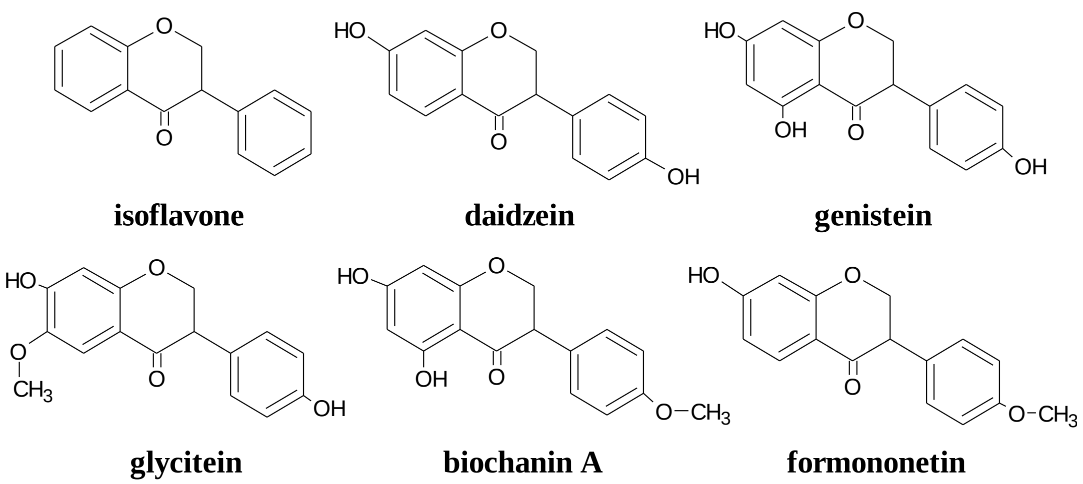


Figure 6: Structures of isoflavones

The basic skeleton of the isoflavonoid molecules (3-phenylchromen-4-one) undergoes various substitutions (methylation, prenylation, hydroxylation, chlorination, attachment of aromatic or aliphatic acids, amino groups, etc.) and cyclizations. Isoflavonoids occur in various oxidation stages, also in the form of dimers and heterodimers (Reynaud et al. 2005).

Based on structural diversity, isoflavones are classified into the following subgroups: isoflavones, isoflavanes, isoflavanchinones, isoflavanones, isoflav-3-ens, rotenoids, dehydrorotenoids, 12a-hydroxyrotenoids, pterokarpans, pterokarpens, 6a-hydroxypterokarpans, kumestans, kumaronochromons, 3-arylkumarins, 2-arylbenzofurans a isoflavanols **see Fig. 7**. The most numerous groups of isoflavonoids are isoflavones and pterokarpans (Veitch 2007).

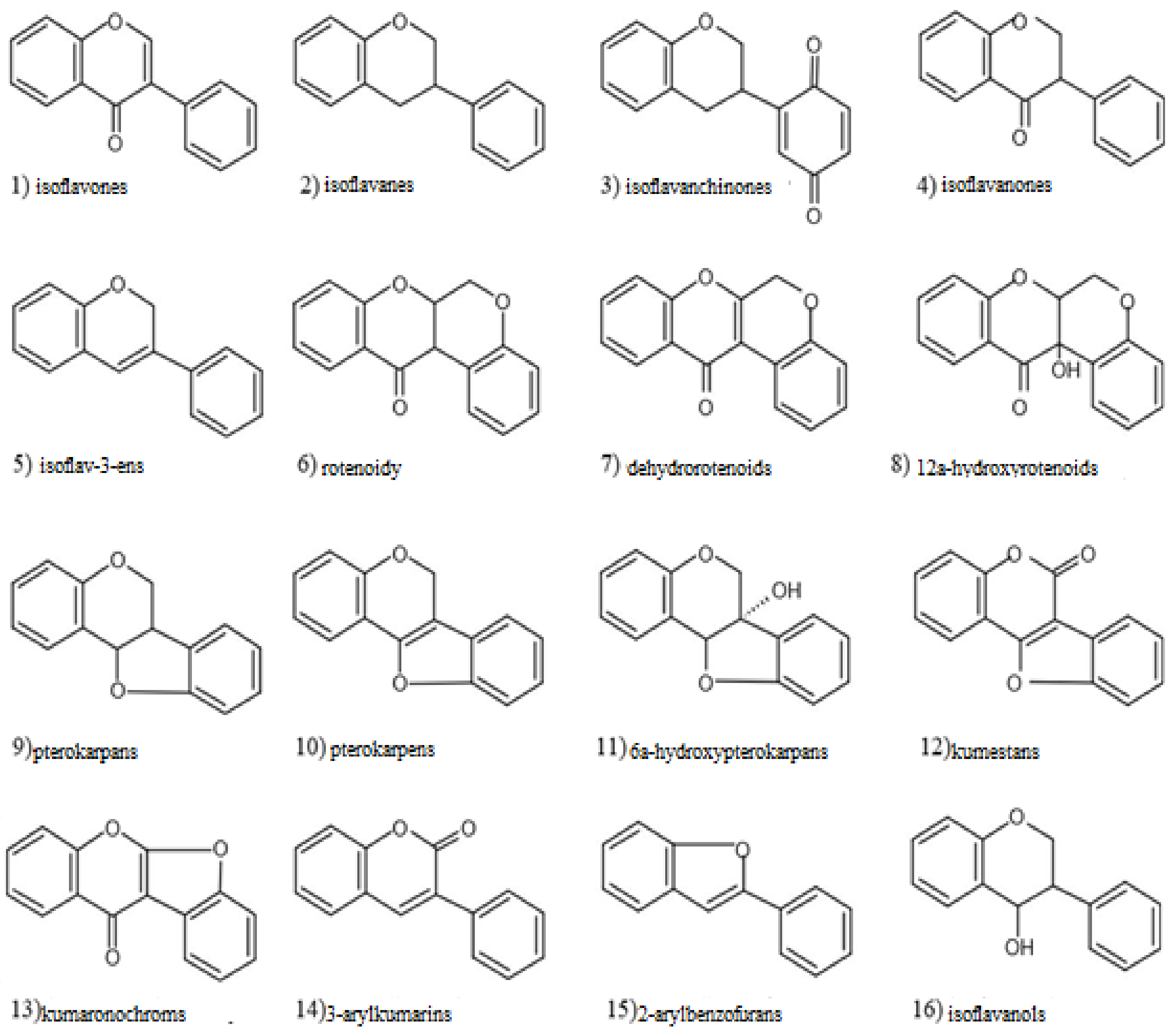


Figure 7: Overview of individual groups of isoflavonoids and their basic chemical structure (Pičmanová 2008; Veitch 2007)

3.3.2. Significance for plants

Isoflavonoids are produced and used mainly to protect against viruses, bacteria, fungi and nematodes. The biosynthetic pathways of isoflavonoid phytoalexins and phytoanticipins are an important potential tool of gene and metabolic engineering for increasing the effectiveness of antimicrobial defense of plants (Dakora & Phillips 1996). For their antimicrobial activity, they have been by phytopathologists included in a group of more than 300 different low-molecular substances, known as phytoalexins and phytoanticipins, which are synthesized by both leguminous and some non-leguminous plants. Phytoalexins react directly in response to pathogen or abiotic stress (heavy metals, UV, herbicides). An example is medicarpin - the main phytoalexin produced by the alfalfa (*Medicago sativa*) as a reaction to infection of pathogenic fungi (Protivová 2006). Phytoanticipins are present in the plant even of the absence of a pathogen. Some isoflavonoids are known for their insecticidal activity (rotenoids) and as insecticides are used in practice (the most important rotenone). In addition to isoflavonoids, a number of non-flavonoid phytoalexins, such as diterpene, sesquiterpene, benzofurans or furanoacetylenes, have been characterized (Reynaud et al. 2005; vanEtten et al. 1994).

The greatest accumulation of isoflavonoids occurs in healthy seeds, roots and rashes and in any organ infected with pathogen (Dakora & Phillips 1996). In the case of foliar infection, the content of phytoestrogens increases. The increase occurs even when attacked by other pests, for example cumestrol accumulates in necrotic spots on the leaves, near the injection of aphids and other insects. In soybeans, significant amounts of genistein, daidzein and their glycosidic conjugates have been found in embryos, uterus, hypokottle, and early roots (Graham 1991). Isoflavonoids are also produced by the root cap cells and released in the root exudates into the soil (Dakora & Phillips 1996).

[redacted] Isoflavonoids and rhizobial symbiosis

Isoflavonoids also play an important role in inducing the symbiotic relationship between plants of the legume family (*Fabaceae*) and diazotropic aerobic bacteria from the rhizobia group (genera *Rhizobium*, *Sinorhizobium*, *Azorhizobium*, *Bradyrhizobium* etc.). Rhizobial symbiosis is of great economic importance because, in line with the current trend of sustainable agriculture, it reduces the requirements of legumes for fertilization with nitrogen fertilizers and also makes the pulp an ideal pre-soil fertility enhancer (Broughton et al. 2000; Spaink 2000).

Rhizobial bacteria synthesize the nitrogenase enzyme catalyzing the reduction of air nitrogen to ammonium cations, a form acceptable to plants (Broughton et al. 2000; Spaink 2000). The symbiosis initiation process involves initial signaling between the plant and its compatible rhizobia type, partner recognition, entry of the bacterium into the root hair, formation of the infectious fiber, and formation of the root tubers necessary to fix the airborne nitrogen. Isoflavonoids produced by plant roots act as chemoattractant for rhizobia and induce expression of bacterial nodulation genes (nodes). Induction leads to the production of Nodic Factors, modified N-acetylglucosamines (chitolipoligosaccharides), which by positive feedback induces the formation of nodules on the root hair of the leguminous plants and the further production of isoflavonoids in the young differentiation zone of the root - elicitor activity principle (Sreevidya et al. 2006; Subramanian et al. 2006).

Among plants in which isoflavonoids have been described and isolated as inducers of rhizobial nod genes are soybean (*Glycine max*), bean (*Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) (Cooper 2004).

3.3.3. Significance for mammals

Besides the undeniable importance for plants themselves, plant isoflavonoids are now regarded as immensely valuable and beneficial compounds to human and other mammals (Cornwell et al. 2004).

Genistein influences several targets in living cells. Due to its structural similarity to estrogen, genistein can bind to estrogen receptors. Daidzein stimulates the growth of estrogen-sensitive breast cancer cells. But more research needs to be specialized on the association between breast cancer risk and daidzein notably before conclusions can be drawn. Daidzein is metabolized in the colon by bacteria to equol and other isoflavones. Daidzein is available as a dietary supplement. Glycitein is unique in that it is an isoflavone found in soy with a methoxy group. Methylated isoflavones have been shown to be more bioavailable and biologically stable than non-methylated isoflavones. Glycitein accounts for 5-10% of the total isoflavones in soy food products. Glycitein shows a weak estrogenic activity, comparable to that of the other soy isoflavones (Kurosu 2011).

Food consumption of isoflavone phytoestrogens has been associated with several benefits for human health **see Tab.1.** (Watanabe 2002). Numbers of studies have shown that isoflavones play an important role in the prevention of distinct cancer forms as breast, colon and prostate cancer, cardiovascular diseases, osteoporosis and alleviate menopausal symptoms (Cornwell et al. 2004). Despite the inconsistency of the published data, there is a clear link between eating rich isoflavonoids and reducing the risk of cancer (Ososki & Kennelly 2003).

Table 1: Isoflavonoid´s benefits on human and animal health (Watanabe 2002)

compound	effect	model species
Daidzein	anticancer, hepatoprotective	rat
	antimenopausal symptoms	women
Daidzin	antialcohol abuse activity	rat
Genistein	anticancer, cardiovascular diseases	human, mouse
	antidiabetic	human
	osteoporosis	mouse
Genistin	anticancer	human
Biochanin A	inhibition of stomach tumor growth	human cell lines

Isoflavonoids also have an antiangiogenic effect and can block uncontrolled cell growth associated with cancer. For example, genistein given to rats reduced their susceptibility to mammary gland cancer and helped them avoid bone loss due to estrogen deficiency (Dixon & Steele 1999).

"*Several studies have reported that isoflavone consumption by postmenopausal women correlated with lower body mass index (BMI), and higher HDL levels (good cholesterol), while a number of studies have also reported absence of beneficial effects of soy on classical metabolic parameters such as bodyweight, serum lipid profiles, fat mass, blood glucose, and insulin profile."* (Cederroth & Nef 2009).

Other studies have examined the effect of phytoestrogens on cognitive function. Men and women received a diet with a high (100 mg/day) and low (0.5 mg/day) content of isoflavonoids. A ten-week study subsequently demonstrated that a soy-rich diet increased both long-term and short-term memory (Cornwell et al. 2004).

However, the potential risks associated with phytoestrogens are also discussed. Isoflavones also exhibit strong estrogenic activity. Like endocrine disruptors (hormone active substances), they also have adverse health effects. They affect, for example, the development of the endocrine system of aquatic organisms. It develops individuals with male and female sexual signs (Vrzáňová & Heresová 2003). Estrogens are responsible for secondary sexual characteristics, sexual organs development and sexual behaviour (Patisaul & Jefferson 2010).

The discovery of estrogenic activity has contributed to the occurrence of "clover disease" of Australian sheep in the 1940s. Observing that female animals living in captivity and fed by soybeans cannot become gravid and fact that sheep grazing on a certain species of red clover are infertile, have led to the consideration that the high content of phytoestrogens in these plants acts in higher animals endogenous estrogens (Vrzáňová & Heresová 2003).

Phytoestrogens enter the animal tissues through grazing or feed. However, exposure to humans cannot be compared with animals. Soy products have about 300 - 500 times higher the phytoestrogen content than the products that replace them. Physiological significance still needs to be examined extensively if it is clearly positive (Suková 2009).

Whether phytoestrogens are beneficial or harmful and to what extent is still unclear. There are contradictory data available in the literature about the undesirable effects of phytoestrogens and the potential health risks associated with their consumption. Their ultimate effect depends on many factors such as route of administration, concentration and dose, individual metabolism, interactions with other drugs, target tissues, receptor types, and last but not least, the effects of phytoestrogens are influenced by endogenous estrogen levels. For example, in an in vitro system, genistein stimulates at physiological doses of 100 nM/l – 1 μM/l proliferation (abundant reproduction) of mammalian cancer cells (Ososki & Kennelly 2003).

[redacted] Metabolism and intake of isoflavones

Isoflavones are synthesized as one group of end-products (iso-flavones) in the phenylpropanoid biosynthetic pathway (Kirakosyan et al. 2007). Isoflavonoids, same as normal estrogens, are absorbed throught the intestine undergo enterohepatic circulation (Zand et al. 1998).

After eating isoflavones rich foods, their biologically inactive glycosides are deglycosylated. In human body, beta-glucosidase is involved in the intestinal microflora and, to some extent, the intestinal hydrolases of the small intestine brush. Bacteria play an important role in the metabolism of daidzein, where are phytoestrogens metabolized by intestinal bacteria to equol (Setchell et al. 2002).

In the colon, equol, a metabolite of daidzein, is produced by bacterial microflora, but only about 30-50% of people have intestinal bacteria for that proces. Equol is a non-steroidal estrogen with his chemical structure very similar to estradiol **see Fig.8**, the endogenous estrogenic hormone, that acts as an anti-androgen by blocking the hormone testosterone for the treatment of menopausal symptoms and prostate enlargement (Kurosu 2011).

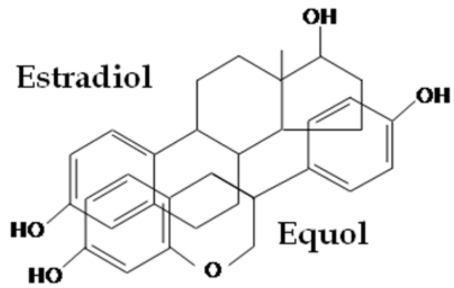


Figure 8: Similarity of equol and estradiol (Salgado & Donado-Pestana 2011)

After the absorption in intestine isoflavones throught enterohepatic circulation are primarily conjugated with glucuronic acid in the liver and then excreted in urine (Zand et al. 1998). The level of daidzein glucuronide is significantly lower in plasma than in urine. The level of daidzein in plasma is approximately 2 times higher (26%) than in urine. Only about 1% of inhaled daidzeine is excreted in the faeces (Ososki & Kennelly 2003).

The intestinal microflora in human metabolism and bioavailability of daidzein has key importance. Metabolites of daidzein occur in human blood plasma, urine and faeces as a result of biotransformation by intestinal microorganisms. In lactating dairy cows, daidzein and other isoflavones are metabolized by rumen microflora, and then the isoflavones and their metabolites are passed into milk (Ososki & Kennelly 2003).

3.3.4. Sources of isoflavonids

The source of isoflavonoids is from most food of plant origin. Highest level of isoflavonoids occurs usually in roots, seedlings and seeds (Matsumura et al. 2013; Abdel-Kader et al. 2008) but are found in less amount in stems, leaves and flowers of older plants. In seeds is the high amount of isoflavonoids glucosyl conjugates - daidzin and genistin (Kirakosyan et al. 2007). The majority of isoflavones, more than 95% of daidzein, glycitein and their respective conjugates, are concentrated in the hypocotyl (Price & Fenwick 1985). While genistein is found both in the hypocotyl and cotyledon, glycitein and its three derivatives are found only in the hypocotyl (Wang & Murphy 1994).

More than 300 plant-producing phytoestrogens are known (Reynaud et al. 2005). The most important producer of *Fabaceae* phytoestrogens is soy (*Glycine max*) containing 50-300 mg per 100 g of net weight (Lapčík 2004). Chickpea contains 0.03 mg/ 100 g dry mass of daidzein, 0.07 mg/ 100 g of genistein, 0.14 mg/ 100 g of formononetin and approximately 1.7 mg/ 100 g of biochanin A. In soy is significantly more daidzein (47 mg/ 100 g dry mass) and genistein (74 mg/100 g dry mass) but less amount of formononetin and biochanin A (Campos-Vega et al. 2010). Different isoflavone contents have also been reported in other legumes such as vigna (*Vigna mungo*), chickpea (*Cicer* sp.), alfalfa (*Medicago sativa*), red clover (*Trifolium* sp.), beans (*Phaseolus vulgaris*), fenugreek (*Trigonella foenum-graecum*), cowpeas (*Vicia faba)*, lentils (*Lens cullinaris*) and cajanus (*Cajanus cajan*) **see Tab.2** (Lapčík 2004; Patel & Barnes 2010). There is few of plant species used as herbal medicines which contain isoflavonoids with estrogenic activity. Kudzu (*Pueraria lobata*) and licorice (*Glycyrrhiza echinata*) are the major source of this isoflavonoids, which are accumulate in roots (Jaganath & Crozier 2010).

Table 2: Content of *Fabaceae* isoflavonoids (Sharma & Ramawat 2016; Kalač 2003)

plant name	isoflavonoid content µg / 100 g dry mass	
	Daidzein	Genistein
Glycine max ("Santa rosa")	56,000	84,100
Glycine max ("Chapman")	41,300	46,400
Vigna mungo	6.9	traces
Cicer arietinum	34.2	69.3
Trifolium pratense	12.200	4.010
Pisum sativum	7.9	22.8
Phaseolus vulgaris	28.2	158
Cajanus cajan	14.6	737
Pueraria lobata (root)	185.000	12.600

Some seeds are also sources of isoflavonoids. This represents sunflower seeds (*Helianthus annuus*) with 0.08 mg / kg of daidzein and 0.14 mg / kg of genistein, peanuts (*Arachis hypogaea*) with 0.50 mg / kg of daidzein and 0.83 mg / kg of genistein, sesam (*Sesamum indicum*), poppy (*Papaver somniferum*), hazelnuts, chestnuts and cocos **see Tab.3** (Velíšek & Hajšlová 2009).

Table 3: Content of daidzein and genistein in selected seed species (Velíšek & Hajšlová 2009; Liggins et al. 2000)

seeds	isoflavonoid content µg / kg dry mass	
	Daidzein	Genistein
peanuts	500	830
sunflower	80	140
poppy	180	70
sesam	37	17
hazelnuts	58	194
chestnuts	79	59
cocos	128	185

To the sources of isoflavonoids we can include certain types of vegetables and fruits as currants and other small fruits, cauliflower, broccoli **see Tab.4** and some other crops with least of isoflavonoids as cereals **see APPENDIX A** (Mazur & Adlercreutz 1998; Ososki & Kennelly 2003; Velíšek & Hajšlová 2009). Small amounts of isoflavones were also detected in cherry, tomato, orange and grapefruit juice (Vítková 2005), in tea and coffee (Mazur & Adlercreutz 1998), in bourbon (Gavaler et al. 1987) and beer **see Tab.5** (Rosenblum et al. 1992). In beer were found in low concentrations (less than 0.1 μM) as barley grain (*Hordeum vulgaris*) and female hops (*Humulus lupulus*) (Lapčík et al. 1998).

Table 4: Sources of isoflavonoids in fruits and vegetables (https://web.vscht.cz/~schulzov/Nutraceutika%20a%20FP/Fytoestrogeny2014.ppt)

fruits and vegetables	content in the sample (μg / g)	
	Daidzein	Genistein
strawberries	0.005	0.046
mandarines	0.003	0.029
mango	0.038	0.032
currant	0.461	1.784
yellow melon	0.015	0.011
raisins	0.590	1.247
broccoli	0.100	0.000
cauliflower	0.050	0.100

In addition to the family of non-legume plants, isoflavonoids (especially daidzein and genistein) were detected in amounts of 5-140 μg per 100 g dry weight in iris (*Iris* sp.). To a lesser extent, isoflavonoids are also present in other taxa: in one family from mosses (*Bryopsida*), three families from conifers (*Pinopsida*), in 10 families from monocots (*Liliopsida*) and 46 families from dicots (*Magnoliopsida*) **see Tab.6** (Macková et al. 2006; Lapčík 2007).

Table 5: The contents of phytoestrogen in beers

(https://web.vscht.cz/~schulzov/Nutraceutika%20a%20FP/Fytoestrogeny2014.ppt)

beer	Daidzein (nmol/l)	Genistein (nmol/l)	Biochanin A (nmol/l)	Formononetin (nmol/l)	Sum (nmol/l)
Bernard 12°	1.30	4.00	II.20	0.50	8.00
Gambrinus 10°	0.79	3.21	3.97	8.46	16.40
Gambrinus 12°	0.41	2.08	2.55	2.00	7.04
Krušovice 12°	0.63	3.05	3.09	1.19	7.96
Martin 10°	0.84	1.96	3.12	0.87	6.79
Martiner 12°	2.54	4.15	3.04	0.59	10.30
Pilsner Urquell 12°	2.05	3.65	4.70	14.40	24.80
Popper 10°	0.75	3.74	3.73	2.33	10.60
Popper 12°	0.20	1.89	1.64	0.16	3.89
Primus 10°	0.64	2.50	2.11	5.79	11.10
Radegast 10°	0.18	0.91	0.53	0.59	2.21
Radegast 12°	0.15	2.15	1.46	0.23	3.99
Radegast Birell	0.21	1.34	1.41	0.51	3.47
Staropramen 10°	0.28	0.96	0.53	0.18	1.95
Staropramen 12°	0.08	0.17	0.82	0.19	1.26
Urpin 10°	0.22	1.42	1.58	0.12	3.34
Velkopopovický kozel 10°	0.56	2.04	0.78	0.64	4.02
Velkopopovický kozel 12°	0.20	1.11	1.42	1.58	4.31

Table 6: Current list of taxons of non-leguminous plants producing isoflavonoids, the numbers of the different structures listed in the table refer only to aglycone (Reynaud et al. 2005; Macková et al. 2006; Lapčík 2007)

class	family	number of structures
Bryopsida:	*Bryaceae*	3
Pinopsida:	*Araucariaceae*	2
	Cupressaceae	8
	Podocarpaceae	5
Liliopsida:	*Asphodelaceae*	1
	Cyperaceae	6
	Eriocaulaceae	1
	Iridaceae	52
	Juncaceae	1
	Liliaceae	4
	Poaceae	1
	Smilaceae	1
	Stemonaceae	3
	Zingiberaceae	2
Magnoliopsida:	*Amaranthaceae*	3
	Apiaceae	4
	Apocynaceae	1
	Aclepiadaceae	12
	Asteraceae	21
	Bombacaceae	1
	Brassicaceae	6
	Cannabaceae	6
	Caryophyllaceae	1
	Celastraceae	1
	Chenopodiaceae	19
	Clusiaceae	3
	Connaraceae	1
	Convolvulaceae	20
	Crassulaceae	1
	Cucurbitaceae	1
	Erythroxylaceae	8
	Euphorbiaceae	3
	Magnoliaceae	1
	Malvaceae	2
	Melastomataceae	1
	Menispermaceae	1
	Moraceae	18
	Myricaceae	2
	Myristicaceae	13
	Myrtaceae	6

Nymphaeaceae	1
Nyctagicaeae	19
Ochnaceae	17
Papaveraceae	2
Polygalaceae	3
Polygonaceae	1
Rhamnaceae	6
Rosaceae	5
Rutaceae	7
Rubiaceae	3
Sapotaceae	2
Scrophulariaceae	6
Solanaceae	1
Sterculiaceae	3
Urticaceae	1
Verbenaceae	1
Vitaceae	1
Violaceae	2
Zygophyllaceae	4

However, there are still discussions about the sources of isoflavonoids in these beverages. Mazur and Adlercreutz (1998) further analysed various leguminous and non-leguminous plants, including the most common fruits, vegetables, cereals, oilseeds and nuts. In some non-leguminous plants, the presence of isoflavones of genistein and daidzein was demonstrated, but in comparation with the isoflavones in leguminous plants was only small amounts **see APPENDIX A**. We can mention the example of root of licorice (*Glychirriza echinata*), which contains an estrogenic isoflavan (glabridin) and isoflavene (glabrene) in small amount (Dixon 2004).

However, there is still little information on the presence of particular phytoestrogens in food, especially in food of animal origin **see Tab.7** and data is missing. It is therefore of great interest to estimate the occurrence of phytoestrogens in cows' milk and products from it, because in animal fattening increases the use of flour from various plant sources which contain large amounts of phytoestrogens (Suková 2009).

In connection with the metabolism of isoflavonoids they have been quantified in plasma, urine, bile and faeces, as well as in human saliva, breast aspirate and prostatic fluid (Morton et al. 1997). They have also been shown to accumulate in breast tissue and milk (Franke et al. 1999) and to cross the blood brain barrier and placenta (Setchell & Cassidy 1999).

Table 7: Total phytoestrogen content in selected food of animal origin (Suková 2009)

foodstuff	isoflavonoids content (μg / 100 g)
whole milk	12
skimmed milk	20
cream	80
goat milk	50
yoghurt	20
cheese gouda	24
cheese camembert	29
butter	11-17
eggs	11
beef	7-19
pork	4-20
fisk and seafood	2-9

[redacted] Milk

Milk is the food of offsprings of all the mammals who bring it to this end. However, cow's milk is also an important source of nutrients and food for human beings for several thousand years. Exploited, absorbed and metabolized phytoestrogens are found in faeces, plasma, cattle urine and they also get into their milk. One factor influencing the concentration of phytoestrogens in milk is the composition of silage. Feeding the clover-grass silos leads to a higher content of isoflavones in milk than when feeding silage containing corn and alfalfa. The genetic composition of dairy cows, their health status, lactation phase, age and season and many other factors influence the composition of milk. Nutrition to dairy cows is one of the most important questions (Jelínek & Koudelka 2003). Incorporation of soy in livestock feed produces the possibility of producing functional foods such as milk and dairy products containing beneficial equols. Such enriched milk could become a source of phytoestrogens and their metabolites. Exploited, absorbed and metabolized phytoestrogens are found in faeces, plasma, urine of cattle, and they also get into their milk. One factor influencing the concentration of phytoestrogens in milk is the composition of silage. Feeding the clover-grass silos leads to a higher isoflavone content in milk than when feeding silage containing maize and alfalfa. The concentration of isoflavonoids is highest in the spring (Mustonen et al. 2009). Consumption of such milk would compensate for the fact that 70 % of the Western European population is not able to fully daidzein metabolization (Jensen 1995).

[redacted]Soy and soy isoflavones

Soy (*Glycine max*) is composed of macronutrients such as lipids (20 %), carbohydrates (40 %) and proteins (40 %). Due to the high protein content, it is a common part of dairy feed and a favorite food in the diet of people, especially on the Asian continent (Cederroth & Nef 2009).

The contents of isoflavones in soybean and soy products have been extensively analysed and studied. Soybeans are a main source of phytoestrogens in the human diet. Some studies have shown that the concentration and composition of isoflavones change considerably, which can be explained by environmental and geographical conditions as well as by the level of industrial transformation. The most abundant isoflavones found in soy and soy products were found isoflavones daidzein, genistein and glycitin, including their glucosides (Shahidi & Naczk 2003). Especially genistein and daidzein as phytoestrogens are the most important and most studied substances contained in soy. In addition, the less known isoflavone is formononetin, which is found only in germinated soy beans and is treated as an exception (Velíšek & Hajšlová 2009).

The total content of isoflavones in soybean is in the range because the isoflavone concentration is dependent on the cultivated variety, maturity, harvest year and climatic and soil conditions (Givens et al. 2008). Data for the total amount range from 47.2 mg to 420 mg / 100 g and can range from 117.6 mg to 174.9 mg per 100 g of soybeans between different growing sites in the same year. Confirming that climatic and soil conditions in soybean cultivation affect the total isoflavone content, it was found that the average daidzein and genistein content in soybean grown in Indonesia is 50% higher than in soybean grown in Australian locations (Shahidi & Naczk 2003).

Soybeans are a rich source of isoflavones, on average, they are reported to contain 2g (from 0.1 - 5 mg total isoflavones per gram of soybean) of isoflavones per kilogram of net weight (Cederroth & Nef 2009). Genistein is generally present at higher levels than daidzein and glycitein (Kurosu 2011).

The total soybean content of isoflavones greatly varies according to the selected product. In soy flour, the total amount of these substances is 120-340 mg / 100 g, in the soy protein isolate 88-164 mg / 100g and in the textured soy protein 66-183 mg / 100g. The highest content of isoflavones contains soybean flour from soy sprout **see Tab.8** (Genovese et al. 2007).

Table 8: Isoflavones (mg/kg) in soy and soy products (Kalač 2003)

product	content in the sample mg / kg
soy (beans)	580 - 3,800
soy flour	830 - 1,780
textured soy protein ("meat")	700 - 1,180
soy milk	35 - 175
tofu	80 - 890
miso	250 - 890

Isoflavones are naturally occurring compounds in foods as glycosides conjugated highly polar conjugated or non-conjugated form. For example, the textured soy protein and tofu have high contents of conjugated isoflavones such as daidzein and genistein, while fermented soy products such as miso, have approximately 90 % of the isoflavones in non-conjugated form **see Tab.9** (Coward et al. 1993). Fermentation can reduce the isoflavones content in food products but increasing its bioavailability. In non-fermented products, isoflavones occur predominantly in the form of glucosides. Fermented products are predominantly aglycans of isoflavones (Hutchins et al. 1995).

Table 9: Content of isoflavones and their derivatives in soya beans and products in mg / kg (Velíšek & Hajšlová 2009)

product	glukosid			aglykon		
	daidzin	genistin	glycitin	daidzein	genistein	glycitein
(soy)beans	234-637	326-888	60-66	10-28	11-30	19-22
flour	147	407	41	4	22	19
isolate	0-88	137-301	34-49	11-63	36-136	25-53
concetrate	0	18	31	0	0	23
tofu	25	84	8	46	52	12
tempeh	2	65	14	137	193	24
soy meat	0-72	96-123	19-21	34-271	93-183	15-54

[redacted] Red clover

Red clover (*Trifolium pratense*), is a high, perennial herb, found abundantly on meadows and on forest light (Deyl & Hísek 2001).

Red clover extract containing isoflavonoids daidzein, genistein, formononetin and biochanin A, due to their high content, has a significant ability to capture free radicals. They act as antioxidants neutralizing the harmful effects of free radicals in the tissues (MyNatureProduct 2018).

Formononetin is found in rumen (in sheep and cow) where is converted into a potent phytoestrogen, equol (Tolleson et al. 2002). Biochanin A exists as isoflavone glycoside. In comparison with other isoflavones, biochanin A is expected to have possible anti-osteoporotic and anticarcinogenic activity Formononetin and biochanin A are the 4'-O-methoxylated isoflavone derivatives and precursors to daidzein and genistein in the metabolic process (Kurosu 2011).

The presence of isoflavonoids in red clover was discovered through so called "Clover Disease" a disorder discovered in grazing livestock. When Australian farmers in the first half of the last century found, in connection with the feeding of this plant, that their sheeps lost their fetus, number of sheeps were unable to be gravid and rams lost their productive power. Farmers found influence of endocrine system on farm animals which has caused excessive intake of estrogenic isoflavones in red clover on pastureland. Therefore, studies have begun to address phytoestrogens, their sources and possible health risks for animals (Lapčík & Stárka 2004).

Isoflavones in red clover have a slightly different composition compared to soy. The red clover contains daidzein, genistein, formononetin, and biochanin A, of which formononetine has the highest proportion of 1-30 g / kg of dry matter versus soybean, where is formononetin considered more like an exception (Velíšek & Hajšlová 2009).

Red clover isoflavones are especially preferred in alternative medicine because they contain four clinically effective isoflavones in pharmacologically significant quantities, whereas soybean has only two - genistein and daidzein. While soy contains only two isoflavones, the red clover has all four (Arndt 2008).

3.4. Dietary habits

Isoflavones have always been part of human diet, but their level in blood plasma could been detected until the year 1980. Before this date, interest in isoflavones and their active metabolites was only marginal. The positive role of isoflavones in the treatment of climacteric syndrome revealed that the incidence of menopausal symptoms, cardiovascular diseases, hormone dependent tumors (tumors in which tumor cells are multiplied due to the presence of sex hormones, such as breast or prostate cancer) and osteoporosis was significantly lower in Asian women than in women with "Western lifestyle" (Allbertazzi et al. 1991). **Tab.10** shows the percentage of women with climacteric syndrome in selected areas. The percentage difference is mainly due to the Far East diet, which is rich in legumes, soybeans and products from it. In Asia, the isoflavonoids intake ranges from 30 to 50 g / day. In western countries is the intake less than 1-2 mg / day (Ransley et al. 2001).

The Western European population is able to fully metabolize these substances only from 30 % in comparison with Asian population, where can convert them more than 60 % of people. The probability of the incidence of tumors in Asia is then 20 times lower (Moon et al. 2006).

Table 10: Percentage of women with climacteric syndrome in selected areas (Barlow et al. 1991**)**

region	percentage of women
USA	85%
Europe	70-80 %
Malaysia	57%
Japan	25%
China	18%
Singapore	14%

Dabrowski and Sikorski (2004) reported that Japanese women had a lower incidence of breast cancer, cardiovascular disease and osteoporosis than women with "Western lifestyle", and Japanese men had a lower incidence of prostate cancer. It is also reported that Japanese women have a lower incidence of menopausal symptoms. We can say that phytoestrogenic isoflavones in soy are attributed to these health benefits (Barlow et al. 1991).

Dietary consumption of isoflavones is well correlated with climacteric symptoms - fewer isoflavones what we receive in the diet, greater are the health problems **see Fig.9** (Ingram et al. 1997).

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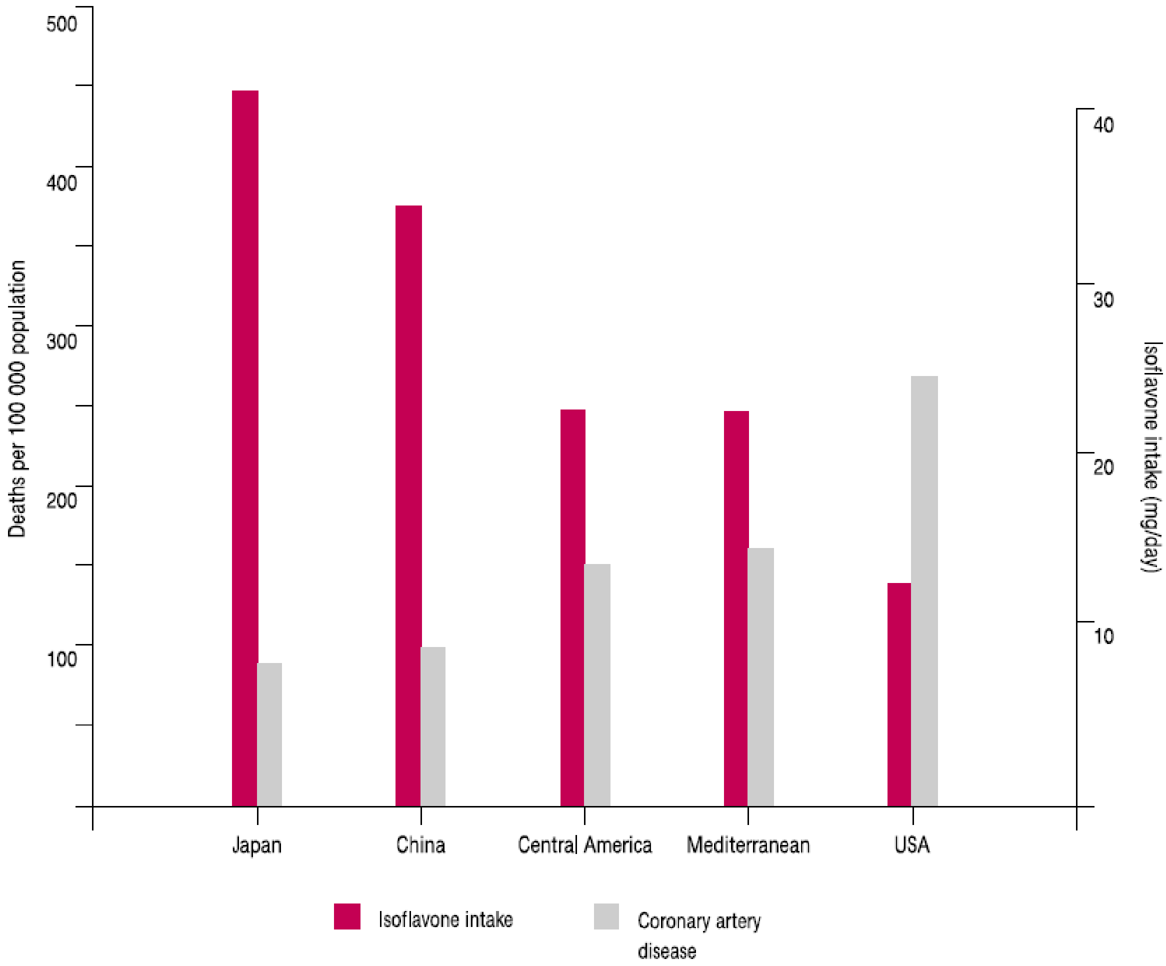

Figure 9: Consumption corralated with climacteric symptoms (Ingram et al. 1997)

The migration of Asians into Western countries, and thus the acceptance of Western diet, increases the incidence of tumor diseases to a similar pattern to the Western population (Ziegler et al. 1993; Whittemore et al. 1995). Study on immigrants from a low-incidence area to a higher incidence area has shown that the incidence of these tumors is more affected by living conditions, the environment, and changing eating habits than genetic factors (Ingram et al. 1997).

A cross-sectional study in Japan in migrating Japanese also showed an increase in cardiovascular disease (Kim et al. 1998) where also inverse relationship between soy consumption and serum total cholesterol was detected (Nagata 1998). Soy may affect cholesterol synthesis, even in newborns and infants. Newborns fed on soy beans have lower cholesterol levels than those fed with maternal or cow's milk (Patisaul & Jefferson 2010).

Most foods containing soy consumed in the Western diet are made from soy proteins that contain glycosidic isoflavones, unlike fermented soy products in which are aglycones (Setchell et al. 2002). Recently, it has been demonstrated in postmenopausal Japanese women that the bioavailability of isoflavones in fermented soy products rich in aglycones is much greater compared to the consumption of glucoside-rich non-fermented soybeans (Okabe et al. 2011). Studies evaluated the bioavailability of daidzein and genistein from soymilk and observed that daidzein was more bioavailable in adult women. The efficiency of absorption of soymilk isoflavones varies from 13 % to 35 % depending on individual gut microflora (Xu et al. 1995).

Soy is consumed in many forms in traditional Asian diets, including soybeans, soybean sprouts, soy milk, tofu, toasted soy protein flours and fermented soybean products such as tempeh, miso, natto, soybean paste and soy sauce (Coward et al. 1993). Soy consumption in most Asian countries is reported to be about per individual 35 g per day and daily intake is between 25 and 100 mg total isoflavones (aglycone equivalents) (Coward et al. 1993; Setchell et al. 2002) and soy protein between 8 and 12 g (Erdman et al. 2004). In Western countries, is more popular consumption of soy in the form of soybean protein products including isolates, grits, flours, concentrates and textured soy proteins (Wang & Murphy 1996) with a daily dose of isoflavones less than 1 mg (aglycone equivalents) (Wei et al. 1995). Higher soy intake is detected in plasma and urinary concentrations, which are higher in Japanese men and women than Western population (Adlercreutz et al. 1991) and are also higher in vegetarians than omnivorous subjects (Adlercreutz et al. 1993).

Total isoflavonoid studies show that the content of isoflavonoids in commonly consumed foods **see Tab. 11**: in bread, cereal products, meat, fish, fruit, vegetables or beer (Andersen & Markham, 2006) is steadily increasing. Total intake is estimated in Japan at 35 mg / day and in Europe is that 1-3 mg of isoflavones per day (Komprda 2008).

Table 11: Estimated intake of flavonoids in different countries (mg / person / day) and main sources of flavonoids in food (Hurst 2008)

state	mg/subject/day	sources of food
Danmark	26	tea, onin, apple
Finland	10 - 41	fruits (apple), vegetables (onion)
Croatia	15	fruits (apple), vegetables (onion)
Italy	23 - 35	red wine, fruits, vegetables
Japan	45 - 68	green tea, soyfood
Netherlands	23 - 33	tea, onion, apple
USA	15 - 23	onion, black tea, apple

4. Discussion

Flavonoids are one of the most important classes of polyphenols. In plant material, they are found to be native, but their concentration can change with different external factors:

The content of phytoestrogens in food depends on a large extent on the cultivar, technological processing of the raw material, its origin and place of production. Another factor can be the storage time of food in shops, climatic and growing conditions, planting and harvesting season or growth-related factors (Moravcová & Kleinová, 2002).

Isoflavonoids pose a great challenge for metabolic engineering:

The biosynthetic branch of isoflavonoids is almost completely described at the molecular level. The individual steps of biosynthesis, the enzymes involved, and the genes encoding these enzymes, are also known. Because of this, isoflavonids appear to be very suitable for gene manipulation at various biosynthetic levels, including the possibility of cloning the respective genes and introducing isoflavonoids into many agriculturally important crops such as wheat, corn or rice which do not naturally produce isoflavones. This would potentially enhance the resistance of these transgenic plants to infections and in addition increase their nutritional value (Dixon 2004).

Despite a number of undeniable evidence of the positive effect of isoflavonoid phytoestrogens on human health, many question still remain:

Studies in the literature have also failed to confirm the positive effect of phytoestrogens. The incidence of "clover disease" in Australian sheep in 1940 ewoked scientists interested in estrogenic effects of isoflavones. Sheep whose diet was mainly composed of clover (*Trifolium subterraneum* L., *Fabaceae*), suffered from reproductive disorders shown as unnatural changes in sexual organs or reducing permanent infertility (Ososki & Kennelly 2003).

Phytoestrogens may negatively affect steroid hormone production:

Is recommend that pregnant women should not include phytoestrogens to their diet. These may disrupt the organization of the female hypothalamus- hypophysis-gonadal axis, which is important in the formation of secondary sexual organs (Baber 2010).

Ibarreta et al. 2001 believe that phytoestrogens could modify the metabolism of sex steroids and cause thyroid hypofunction.

Some scientists postulate that phytoestrogens may have evolved to protect the plants by interfering with the reproductive ability of grazing animals:

It has been hypothesized that plants use phytoestrogens as part of their natural defence against the overpopulation of herbivore animals by controlling female fertility. Because plants cannot escape attack and that many plants occur as unintended food sources, inhibition of herbivore reproduction on the same place would reduce their numbers and thus reduce the degree predators on the plant or their genetically similar kinds or their offsprings (Hughes 1988).

5. Conclusions

Isoflavonoids, due to their wide range of biological effects, belong to intensively studied secondary metabolites of plants. They are currently under constant scientific research. Until now, the most studied isoflavonoids are: daidzein, genistein, glycitein, formononetin and biochanin A.

Their producers are mainly the plants from family of leguminoses (*Fabaceae*), as soy (*Glycine max)*, vigna (*Vigna mungo*), chickpea (*Cicer* sp.), alfalfa (*Medicago sativa*), red clover (*Trifolium* sp.), beans (*Phaseolus vulgaris*), fenugreek (*Trigonella foenum-graecum*), cowpeas (*Vicia faba)*, lentils (*Lens cullinaris*) and cajanus (*Cajanus cajan*). The growing sensitivity of analytical methods makes it possible to isolate and identify isoflavonoids from other plant families. At present, a further 59 isoflavonoid producing families are known. They were found in 1 family from mosses (*Bryopsida*), 3 families from conifers (*Pinopsida*), 10 families from monocots (*Liliopsida*) and 46 families from dicots (*Magnoliopsida*). Isoflavonoids also occur in some fruits, vegetables and certain seeds. Include currants where the proportion of isoflavonoids is highest with predominance of genistein, strawberries, mandarins, mango, rainsins and cauliflower, broccoli or peanuts, poppy, sunflower seeds, sesam seeds, hazelnuts, chestnuts or cocos. Among the representatives of isoflavonoids with healing effects we can include kudzu (*Pueraria lobata*) and licorice (*Glycyrrhiza echinate*), which have isoflavonoids stored in roots. But it is very presumably that the number of producer's families is actually larger. Traces of isoflavonoids were also found in beverages like beer, bourboun or green and black tea. The major source of isoflavonoids is soy and soy products. Another major source of isoflavones is the red clover, which contains most of the compounds mentioned above – daidzein, genistein, formononetin a biochanin A, whereas soy contains only daidzein, genistein and in ungerminated beans formononetin.

In isoflavonoids was found endogenous estrogenic activity. Because of this activity, they are called phytoestrogens, which are plant hormones similar to animal hormone - estrogen. Their similarity is related to similar biological effects both on plants and on animals. In plants, they either act as a direct response to pathogens or are permanently resistant to pathogens in plants. They also act as chemoattractants for tuber bacteria in the process of rhizobial symbiosis. This knowledge is applied in practice in agriculture to streamline agricultural land or to natural protect of plants from pathogens. In the animal kingdom, they can affect the fertility. Throughout their production of milk, the effects of isoflaonoids on humans can also be adversely affected. In human isoflavonids work as prevention of menopausal symptoms, reduce the risk of osteoporosis, cardiovascular diseases, and also prevent breast, prostate or intestinal cancer.

Menopausal risks are much more common in Western-style women than in Asian women. The important role can play processing of products if they are fermented or nonfermented. The bioavability in soy food is higher in fermented products (tempeh, miso, natto etc.) with aglycones form than in nonfermented (soy protein, flour etc.) foods with glycosides form and Western people consume more nonfermented foods against Asian people consuming most of fermented food. In the Far East, there is also a lower incidence of hormone-dependent cancers. These differences are attributed to Asian diets rich in isoflavones. Asian intake of isoflavonoids is 30 times higher than their intake in Western countries. A lesser incidence of diseases may also be related with the ability to metabolize the given isoflavonids. Isoflavonoids are metabolised in the intestines by intestinal microflora. It has been found that the Asian population does not have the problem of absorbing these substances, unlike Western cultures, where only 30 % of the population has this ability. Metabolites absorbed in the intestines then appear in blood plasma, urine and faeces as a result of biotransformation by intestinal microorganisms. The content of these metabolites in the plasma should be lower than in the urine, which is excreted their excess amount in the body. Thus, the content of isoflavonoids in the body can affect the diseases mentioned above.

Human health is influenced by isoflavonoids more by geographical area connected with local diet than genetic information. It was proved that in immigrants from Asia, after their change of domicile, begin have the same problems as western people – their incidence of cancers began increase.

Other species should be more studied. I think a lot of isoflavonoids which have not been discovered yet. It is necessary to find more plant species to identify new isoflavonoid compounds and determinate whether their effects are positive or negative on plants, human or animals.

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Appendices

List of the Appendices:

Appendix A: Isoflavones levels in various food sources (in nmol/g dry weight) (Mazur & Adlercreutz 1998)

common name of plant species	Daidzein	Genistein
soybean	413-2,205	993-3,115
kidney bean	<1-2	<1-19
chickpea	<1-8	3-8
pea	<1	<1
lentil	<1	<1
kudzu root	7,283	467
peanut	1	2
sunflower seed	<1	<1
sesame seed	6	<1
flaxseed	0	0
wheat bran	<1	<1
barley (whole grain)	<1	<1
rye	0	0
strawberry	0	0
cranberry	0	0
blueberry	0	0
raspberry	0	0
broccoli	<1	<1
red cabbage	<1	<1
zucchini	0	0
carrot	0	0
garlic	0	0
beetroot	0	0
black tea	trace	trace
green tea	trace	trace