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SYMPOSIUM

The role of antioxidant phytonutrients in the prevention of diseases+

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ABSTRACT The authors investigated the *in vitro* antioxidant properties of nine selected flavonoid aglycons, namely quercetin, kaempferol, myricetin, apigenin, luteolin, daidzein, genistein, formononetin and biochanin A. The *in vitro* antioxidant power of flavonoids basically depends on their chemical structure. The concentration of those flavonoids in vegetables and fruits frequently consumed in Hungary was examined by a RP-HPLC method. Referring to the total flavonoid content, rich sources were onions, parsnip, spinach, different parts of celery, and lentils. Among the fruits berries were very rich sources of flavonoids (blackberry, red current). The flavonoid intake was estimated in two groups, first included more than 500 schoolboys and girls aged 12-15 years, and the second group was about 200 healthy adults aged 25-60 years. The average dietary flavonoid intake of the children and the adults was 19.5±26.6 and 18.8±28.9 mg/day, respectively. The flavonoid intake showed high differences among the subjects. The consumption of flavonoids in the children's group was from 0 to 179 mg/day, and in the adults¢ group from 0.5 to 310 mg/day. Although two groups in our study did not represent exactly the total Hungarian population it is supposed that the average daily flavonoid intake of the population is not different from these data. The estimated Hungarian intake is very similar to that of others published in the literature.
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KEY WORDS

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The antioxidant characteristics of plant derived materials can be attributed to their content of polyphenols. Until recently, most of the nutritional interest in polyphenols was in the deleterious effects caused by the ability of polyphenols to bind and precipitate macromolecules, such as dietary protein, carbohydrate, and digestive enzymes, and therefore reducing food digestibility. However, interest in food phenolics has increased, because of their antioxidant and free radical scavenging abilities. Polyphenols constitute one of the most numerous and widely distributed group of substances in the plant kingdom, with more than 8000 phenolic structures currently known (Harborne 1993). Polyphenols are products of secondary metabolism of plants and ubiquitous in all plant organs. They arise biogenetically from two main synthetic pathways: the shikimate and the acetate pathway (Harborne 1993; Bravo 1998).

Natural polyphenols can range from simple molecules, such as phenolic acids, to highly polymerised compounds, such as tannins. They occur primarily in conjugated form, with one or more sugar residues linked to hydroxyl groups, although direct linkages of the sugar unit to an aromatic carbon atom also exist. According to Harborne (1989) polyphenols can be divided into at least 10 different classes depending on their basic chemical structure. Flavonoids, which constitute the most important single group, can be further subdivided into 13 classes, with more than 4000 compounds described until 1990 (Harborne 1989, 1993). Compounds most widely occurred in the nature are flavonols, flavones, flavan-3-ols, isoflavones, flavanones, flavanols, anthocyanidines and proanthocyanidines (Bravo 1998).

Flavonoids are almost ubiquitous in plant foods (vegetables, cereals, legumes, fruits, nuts, etc.) and beverages (wine, cider, beer, tea, cocoa, etc.). The presence of flavonoids in plant foods is largely influenced by genetic factors and environmental conditions. Other factors such as germination, degree of ripeness, variety, processing, and storage also influence the content of plant phenolics (Herrman 1976, 1988; Mazza 1995; Peleg et al. 1991). Flavonoids and other polyphenols are partially responsible for sensory and nutritional qualities of plant foods. The astringency and bitterness of foods and beverages depends on the content of polyphenols.

Many flavonoids and polyphenols can exhibit antioxidant activity as their extensive, conjugated π-electron systems allow ready donation of electrons, or hydrogen atoms, from the hydroxyl moieties to free radicals (Bors et al. 1987). However, the antioxidant efficacy, in terms of reaction stoichiometry (the number of radicals which one phenolic molecule may annihilate) and reaction kinetics (the rate at

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+In memory of Professor Béla Matkovics

which radicals are annihilated), may vary considerably. This will depend on structural features, such as the number and positions of the hydroxyl moieties on the ring systems, and the extent by which the unpaired electron in the oxidised phenolic intermediate can delocalise throughout the molecule. Most polyphenols, especially flavonoids are very effective scavengers of hydroxyl and peroxyl radicals (Manach et al. 1996). Flavonoids are chelators of metals and inhibit the Fenton and Haber-Weiss reactions, which are important sources of active oxygen radicals (Shahidi and Wanasundara 1992). In addition, flavonoids retain their free radical scavenging capacity after forming complexes with metal ions (Afanas'ev et al. 1989).

Interest in food phenolics has increased owing to their role as antioxidants, antimutagens, and scavengers of free radicals and their implication in the prevention of pathologies such as cancer and cardiovascular disease. Epidemiological studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of cardiovascular disease and certain types of cancer (Hertog et al. 1993, 1995; Hertog 1996; Rimm et al. 1996; Hollman and Katan 1999). Food products from fruits and vegetables are important part of a well-balanced, healthy diet in humans.

Flavonols, flavones and isoflavones constitute three major subclasses of flavonoids (Fig 1). Flavonols and flavones have similar C-ring structure with a double bond at the 2-3 position. Flavones, as opposed to flavonols, lack a hydroxyl group at the 3-position. Major flavonols are quercetin (3,5,7,3',4'-pentahydroxyflavone), kaempferol (3,5,7,4'-tetrahydroxyflavone), and myricetin (3,5,7,3',4',5'-hexahydroxyflavone). The most abundant flavones in plants are luteolin (5,7,3',4'-tetrahydroxyflavone) and apigeo=n (5,7,4'-trihydroxyflavone). B-ring of isoflavonoids is connected to 3-C. Most frequently occurred isoflavonoids are isoflavones with a double bond between 2- and 3-C. Major isoflavones are genistein (5,7,4'-trihydroxyisoflavone), daidzein (7,4'-dihydroxyisoflavone), formononetin (7-hydroxy-4'-methoxyisoflavone) and biochanin A (5,7-díhydroxy-4'-methoxyisoflavone).

In present study the *in vitro* antioxidant properties of some selected flavonoids were investigated, the flavonoid composition of plant foods was measured, and based on these analytical data the flavonoid intake in two groups of the Hungarian population was estimated.

Materials and Methods

Chemicals

Quercetin, luteolin, myricetin, kaempferol, genistein, daidzein, formononetin, biochanin A and t-butylhydroquinone were purchased from Sigma, apigenin from Fluka and methanol of chromatography grade were obtained from Merck. All other chemicals and reagents were of analytical grade from Reanal (Hungary).

Fresh and dried fruits

45 selected fresh and dried fruits and 31 vegetables (1 kg, or a minimum of three units) were purchased from 3 different greengrocers in the local markets in Budapest at a period of their most frequent consumption. The edible parts of the fruits and vegetables were used to the examination, and samples from three locations were combined. After buying the samples were immediately cleaned, chopped into small pieces and freeze-dried. After lyophilization, samples were allowed to equilibrate in open air and ground to pass a 0.5-mm sieve. Moisture was measured by drying at 105ºC. The food samples were stored at -18ºC for less than 4 months until analysed.

Hydrogen-donating ability

Hydrogen-donating ability of pure flavonoids dissolved in methanol was determined in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical as described Blois (1958) and modified by Hatano et al. (1988). Hydrogen-donating ability is expressed as I_{50}, the amount of the sample that is needed for 50% discoloration of DPPH. The lower the value the higher the activity.

Reducing power

Reducing power of methanolic solution of flavonoids was determined according to the method of Oyaizu (1986). Diluted and/or filtered sample (1 ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml); the mixture was incubated at 50ºC for 20 min. Trichloracetic acid (2.5 ml, 10%) was added to the mixture which was then centrifuged at 1.500 g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and freshly prepared $FeCl_3$ solution (0.5 ml, 0.1%). The absorbance was read at 700 nm. Increased absorbance of the reaction mixture indicates elevated reducing power. Reducing power is given in ascorbic acid equivalent (ASE ml^{-1}) that shows the amount of ascorbic acid expressed in mmol those reducing power is the same than that of 1 ml sample.

Total antioxidant status

This spectrophotometric technique measures the relative abilities of antioxidants to scavenge the $ABTS^{\cdot+}$ [2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate)] in comparison with the antioxidant potency of standard amounts of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The measurement is based on the procedure described by Miller et al. (1993). The radical cation $ABTS^{\cdot+}$, produced by the ferrylmyoglobin radical generated from methmyoglobin and H_2O_2 in the presence of peroxidase, is a blue-green chromogen with characteristic absorption at 660 nm. In the presence of antioxidants absorbance is decreased.

The determination of total antioxidant status (TAS) of flavonoids was carried out using the Randox diagnostic kit with a COBAS MIRA automatic laboratory analyser.

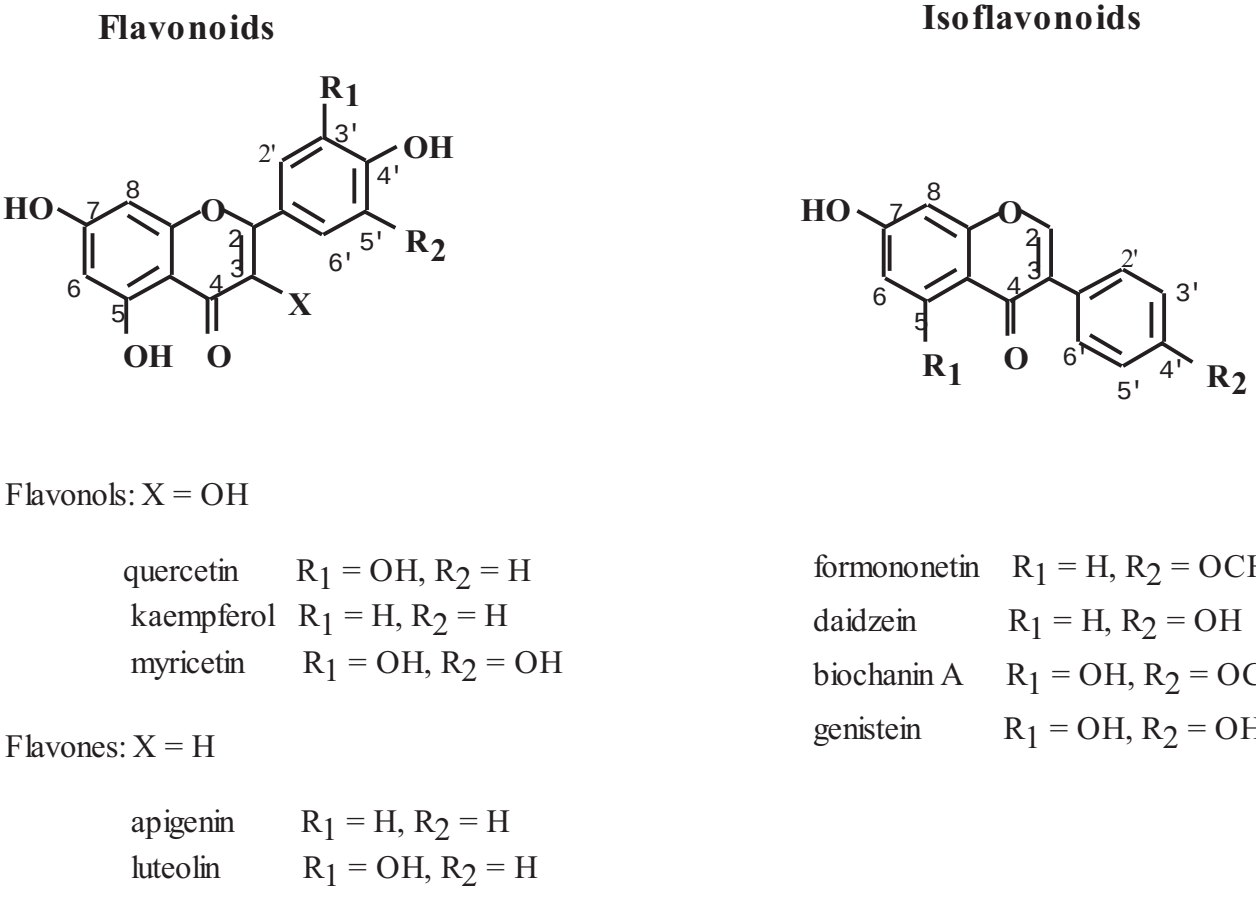
HPLC analysis of flavonoids

The flavonols (quercetin, kaempferol, myricetin) and the flavones (apigenin, luteolin) were measured as aglycons according to Hertog et al. (1992). Briefly, flavonoid glycosides were extracted and hydrolysed to their aglycons with 2.0 M HCl in boiling 50% aqueous methanol in the presence of 0.1 g t-butylhydroquinone for two hours. After refluxing the extract was allowed to cool and was subsequently made up to 50 ml with methanol and sonicated for 5 min. Approximately 2 ml was filtered through 0.45 μm filter (Chromafil AO-20/25) before injection. The resulting aglycons were quantified by RP-HPLC (Perkin Elmer) on a Premisphere C_{18} column (150 x 3.9 mm, 5 μm, Phenomenex, USA) using methanol/phosphate buffer (45/55 v/v, pH 2.4), as a mobile phase and UV detection (370 nm).

Limit of detection was defined as the amount of flavonoids resulting in a peak height of 3 times higher than the standard deviation of the baseline noise. Peak identification was confirmed with the use of known retention time of pure flavonoids. Quantification of the flavonoids was by peak area measurement. Calibration curves of individual flavonoids were made over a range of 1-8 μg/ml. Detector response was linear over the concentration range used. For all standards r^2 was higher than 0.998.

Estimation of dietary intake of flavonoids

Data of flavonoids come from the analytical results were built into an existing computer system used for calculation of the intake of different nutrients. Based on these data the flavonoid content of more than 2600 meals was calculated. Estimation of flavonoid intake in two groups of the Hungarian population was done according to the international standard method, a three-days dietary record. Persons involved in the study were asked to note everything that they consumed during three different days (2 non-consecutive weekdays, 1 weekend day). Avoiding the incorrect data qualified dieticians helped and interviewed the subjects. The flavonoid intake was estimated in two groups, first included



Flavonols: X = OH

quercetin	$R_1 = OH, R_2 = H$
kaempferol	$R_1 = H, R_2 = H$
myricetin	$R_1 = OH, R_2 = OH$

Flavones: X = H

apigenin	$R_1 = H, R_2 = H$
luteolin	$R_1 = OH, R_2 = H$

formononetin	$R_1 = H, R_2 = OCH_3$
daidzein	$R_1 = H, R_2 = OH$
biochanin A	$R_1 = OH, R_2 = OCH_3$
genistein	$R_1 = OH, R_2 = OH$

Figure 1. Chemical structure of flavonoids and isoflavonoids.

Table 1. *In vitro* antioxidant activity of different flavonoids.

Family	Molecule	Hydrogen donating ability[1], I_{50} (mg)	Reducing power[2] (ASE/mg)	TAS[3] (mmol/g)
Flavonols	**Quercetin**	7.3	15.0	92.6
	Myricetin	9.9	15.3	98.7
	Kaempferol	19.0	9.3	8.3
Flavones	Luteolin	7.2	9.9	16.5
	Apigenin	>390	0.73	4.3
Isoflavonoids	Genistein	1441	0.130	8.85
	Daidzein	1588	0.060	1.98
	Biochanin A	672	0.117	2.30
	Formononetin	4254	0.028	1.32

[1]I_{50} expressed in mg is the amount of the molecule that is needed for the 50% discolouration of DPPH radical.
[2]Reducing power is given in ascorbic acid equivalent (ASE) that shows the amount of ascorbic acid expressed in mmol those reducing power is the same than that of 1 ml sample. [3]Total antioxidant status (TAS) is measured by the Randox Kit and the characteristic is expressed in Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent.

521 schoolboys and girls aged 12-15 years, and the second group was 204 healthy adults aged 25-60 years.

Results and Discussion

In vitro antioxidant activity of flavonoids

Flavonol and flavon molecules exhibited strong antioxidant properties in different *in vitro* systems (Table 1). The flavonol quercetin and myricetin, and the flavon luteolin were effective hydrogen-donating molecules in the presence of DPPH radical, because they had low I_{50} value that is the amount of the molecule needed for the 50% discolouration of the DPPH radical. As it can be seen on Figure 1 quercetin and luteolin have two OH groups on B ring, while myricetin has three, and kaempferol and apigenin have only one. Luteolin and apigenin do not have OH group at C-3 position. As Bors and co-workers (1990) established based on their studies with pulse radiolysis three structural elements are essential for the strong antioxidant property of a flavonoids: 1) the *ortho*-dihydroxy (catechol) structure in the B-ring, the obvious radical target site for all flavonoids with saturated 2,3-double bond (flavan-3-ols, flavanones, cyanidin chloride); 2) the 2,3-double bond in conjunction with a 4-oxo function; and 3) the additional presence of both 3- and 5-OH groups for a maximal radical scavenging potential (Fig. 2). Results of reducing power and TAS also reflect these connections between the chemical structure and antioxidant property. In this *in vitro* system quercetin was the most effective antioxidant while apigenin was the weakest compound. For the effective antioxidant property a resonance stable chemical structure is essential as it is realized in the case of quercetin (Fig. 3).

Isoflavonoids also expressed antioxidant properties but these were much lower than those of flavonols and flavones (Fig. 1). B-ring of the isoflavonoids is connected with 3-C instead of 2-C unlike in flavonoids. From isoflavonoids studied here genistein and biochanin were more effective than daidzein and formononetin. It is clear that both hydroxy groups in the 4' and 5 position are needed for antioxidant activity of the molecule as in genistein. Biochanin A have similar activity, OCH_3 group at 4' position do not modify considerably the antioxidant property. According to TAS result genistein is stronger than apigenin but the two other parameters as H-donor ability and reducing power show opposite property. The resonance-stabilized quinoid structures of isoflavones show that the carbonyl group at position 4-C loses its functionality (Shukla et al. 1997) thus explaining the similar or a bit stronger antioxidant activity of genistein compared with apigenin.

Flavonoids and isoflavonoids exhibited significant antioxidant properties in different *in vitro* tests. Antioxidant effectiveness of a compound is significantly dependent on its chemical structure. However, it is very important to take into account that chemical structure of the compounds could dramatically change during metabolism. Functioning of intestinal bacteria and different enzymes, hepatic/microsomal transformations that are hydroxilation, methylation, reduction, conjugation, can lead to a new compound having different antioxidant properties from the original molecule. Therefore the results of *in vitro* studies have to be managed in a circumspect way.

Flavonoid composition of plant foods

Flavonol and flavon content of plant foods is strongly influenced by extrinsic factors such as variation in plant type

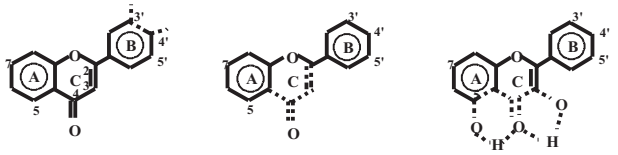


Figure 2. Structural elements of flavonoids responsible for the antioxidant properties.

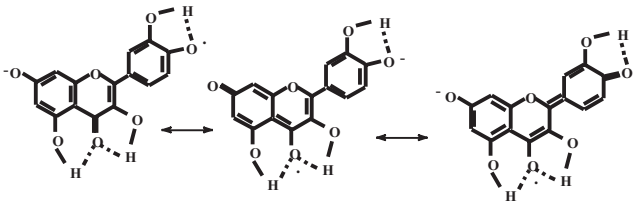


Figure 3. Resonance stable structure of quercetin.

and growth, season, climate, and degree of ripeness (Hollman and Arts 2000; Stewart et al. 2000; Parr and Bolwell 2000). Flavonoid composition of some fruits and vegetables investigated in this study is presented in Table 2. More detailed data are published elsewhere (Lugasi and Hóvári 2000, 2002).

Quercetin and kaempferol are proved to be the most widespread flavonoids in vegetables. Quercetin levels in the edible parts of most vegetables were generally below 10 mg/kg. The highest quercetin concentration could be detected in spinach (272.2 mg/kg) and in the different types of onion (67.1-171.3 mg/kg). High quercetin level was also found in dill (74.5 mg/kg), crisped lettuce (35.0 mg/kg), and broccoli (15.4 mg/kg). Very low level or no quercetin was detected in different root vegetables such as celery and beetroot, radishes and also in *Brassica* vegetables except broccoli. Significant amount of kaempferol was observed in parsnip (66.4 mg/kg), leek (45.8 mg/kg), fresh onion collected in early summer (34.3 mg/kg), and broccoli (30.8 mg/kg). Average level of flavonoid could be detected in other *Brassica* and root vegetables. Practically there was no kaempferol in leafy vegetables except crisped lettuce (8.4 mg/kg). The flavonol myricetin only in five vegetables was found, namely Swedish turnip (85.4 mg/kg), parsley leaves (80.8 mg/kg), celery leaves (43.4 mg/kg), lettuce (10.2 mg/kg), and dill (7.0 mg/kg). Regrettable the leaves of parsley, celery and dill are consumed as a condiment in special Hungarian dishes therefore the participation of these vegetables in the flavonoid intake of the population is probably negligible. Significant amount of flavon luteolin could be detected in celery leaves (111.4 mg/kg), spinach (66.4 mg/kg), red beet (18.3 mg/kg), kohlrabi (13.0 mg/kg), and different types of pepper (10.7-11.3 mg/kg). Apigenin was detected in celery leaves (248.0 mg/kg), Swedish turnip (154.0 mg/kg), and celery root (24.1 mg/kg).

In present investigation all of vegetables had significant amount of flavonols and/or flavones. The best sources of selected five flavonoids were spinach (338.6 mg/kg), Swedish turnip (265.3 mg/kg), red, young and old onions (195.6,

Table 2. Flavonoid content of some plant food (mg/kg fresh weight).

Sample	Quercetin	Kaempferol	Myricetin	Luteolin	Apigenin	Total flavonoids
Vegetables						
Broccoli	15.4	30.8	nd*	nd	nd	46.2
Kohlrabi	4.0	24.3	nd	13.0	nd	41.3
White cabbage	1.6	11.9	nd	4.2	nd	17.7
Red onion	121.5	2.6	nd	nd	nd	124.1
Purple onion	171.3	24.3	nd	nd	nd	195.6
Pepper	9.4	nd	nd	10.7	nd	20.1
Crisped lettuce	35.0	8.4	nd	3.9	nd	47.3
Spinach	272.2	nd	nd	66.4	nd	338.6
Dill	74.5	nd	7.0	nd	nd	81.5
Parsley leaves	nd	nd	80.8	nd	nd	80.8
Celery leaves	nd	nd	43.4	111.4	248	402.8
Celery root	1.8	nd	nd	nd	24.1	25.9
Swedish turnip	3.2	22.7	85.4	nd	154.0	265.3
Horse radish	5.7	25.7	nd	9.0	nd	40.4
Pumpkin	nd	nd	nd	16.3	nd	16.3
Fruits						
Water-melon	nd	nd	nd	18.4	nd	18.4
Musk-melon	nd	nd	nd	25.8	nd	25.8
Sour cherry	29.2	nd	nd	nd	nd	29.2
Mulberry	24.7	nd	452.6	nd	nd	477.3
Blackcurrant	52.8	nd	nd	nd	nd	52.8
Blackberry	14.0	nd	636	nd	nd	650
Strawberry	9.0	nd	994	nd	nd	1003
Grape (Saszla)	38.7	nd	nd	nd	nd	38.7
Walnut	nd	nd	4565	nd	nd	4565
Kiwi	nd	nd	nd	22.3	nd	22.3
Banana	nd	nd	22.8	nd	nd	22.8
Apple	38.3	nd	nd	27.0	nd	65.3
Pear	24.7	nd	nd	nd	nd	24.7
Plum	23.3	nd	nd	nd	nd	23.3
Apricot	11.5	nd	nd	nd	nd	11.5

*nd – under the detection limit

124.1, and 101.6 mg/kg, respectively), and celery leaves (154.8 mg/kg). As the formation of flavonoids is light-dependent, flavonoids occur predominantly in the leaves. In contrast, the concentration flavonoids is low -<1 mg/kg fresh weight- in roots or tubercules; in some cases these compounds may accumulate in the underground parts of certain plants such as onions and radishes. There could be not found any vegetables free from flavonoids.

From the results of HPLC analysis basically became clear that fruits frequently consumed in Hungary did not contain kaempferol and apigenin at all (Table 2). None of five measured flavonoids was detected in any varieties of grapes (Big-grained, Othello), green gooseberry, peach, quince-pear, grapefruit, orange, tangerine and poppy-seed, in oily nuts such as almond, pistachio, kasewnut, groundnut, hazel-nut and coco-nut, and in dried fruits such as raisin, date, fig, and prunes.

Stone fruits have low level of flavonoids except for walnut which is not a really fruit but an oily crop. Only quercetin could be detected in plums and apricot at a concentration range of 11-23 mg/kg. Extremely high level of myricetin was found in walnut (4565 mg/kg), but other flavonoids were not present. Berry fruits seem to be very rich sources of flavonoids. Quercetin was found in sweet and sour cherry (8.9 and 29 mg/kg), in gooseberry (9.1 mg/kg), in strawberry (9.7 mg/kg), in blackberry (14.5 mg/kg), in mulberry (24.7 mg/kg) and blackcurrant (52.8 mg/kg). Extremely high concentration of myricetin was observed in some berries such as redcurrant, mulberry, blackberry, and strawberry (42.9, 452.5, 636, and 993.6 mg/kg, respectively). Fruits similar to apple have luteolin and quercetin at a concentration around 20-30 mg/kg. Two varieties of apple contained quercetin (Gála 30.1 mg/kg, Golden 38.3 mg/kg) and two others luteolin (Golden 27.0 mg/kg, Jonatán 22.5 mg/kg). Pomegranate has also luteolin (18.9 mg/kg). Quercetin was found also in pear (24.7 mg/kg). Water-and muskmelon are very popular fruits in Hungary; they are frequently consumed especially in August. As it can be seen in Table 4 only luteolin was detected in water- and muskmelon and pumpkin (18.4, 25.8 and 16.3 mg/kg, respectively). Among citrus fruits only lemon has flavone luteolin at a concentration of 23.1 mg/kg edible part. From other exotic fruits kiwi contains also luteolin (22.3 mg/kg) and banana has myricetin (22.8 mg/kg).

Estimation of the flavonoid intake in two groups of the Hungarian population

The flavonoid intake was estimated in two groups, first included more than 500 schoolboys and girls aged 12-15 years, and the second group was about 200 healthy adults aged 25-60 years. The average dietary flavonoid intake of the children and the adults was 19.5±26.6 and 18.8±28.9 mg/day, respectively (Table 3). There was no difference between the two groups. At the same time the flavonoid intake showed high differences among the subjects. The consumption of flavonoids in the children's group was from 0 to 179 mg/day, and in the adults¢ group from 0.5 to 310 mg/day. Opposite of the literary data where the quercetin was said to be the most frequently consumed flavonoid, in our study this compound was myricetin. Myricetin represented 44 - 57% of the total flavonoids while quercetin was 28.6 – 36.1%, and the proportion of kaempferol, luteolin and apigenin was lower than ten percent. With the use of the computing system the average dietary intake of the nutrients such as fat, carbohydrate, protein, energy, micro and macro elements, vitamins, fibre, fatty acids, cholesterol etc. was also calculated in the studied groups (results are not shown). These data were compared to those were calculated during the first representative (in 1985-88) and second (in 1992-94) Hungarian nutrition survey (Bíró 1992; Bíró et al. 1996). These studies emphasized that the daily intake of fat, cholesterol and sodium is very high, while the consumption of other important compounds such as fibre, vitamins, microelements was not high enough. The elements of the improper nutritional habit could be observed in present study. Although two groups in our study did not represent exactly the total

Table 3. Flavonoid intake in two groups of the Hungarian population.

Adults	Quercetin	Kaempferol	Myricetin	Luteolin	Apigenin	Total flavonoids
Number of subjects	204	204	204	204	204	204
Average	6.38	1.12	8.31	1.66	0.85	18.80
SD	6.51	1.51	26.35	2.42	0.87	28.90
Minimum	0.28	0	0	0	0	0.50
Maximum	40.41	14.22	298.9	10.65	5.5	309.67
Children						
Number of subjects	521	521	521	521	521	521
Average	5.56	0.67	11.18	1.55	0.57	19.60
SD	5.54	0.97	25.51	2.24	0.71	26.66
Minimum	0	0	0	0	0	0
Maximum	40.25	11.18	175.9	15.89	4.18	179.35

Hungarian population it is supposed that the average daily flavonoid intake of the population is not different from these data. The estimated Hungarian flavonoid intake is lower than the Dutch (23 mg), Danish (28 mg) and Finnish (55 mg) data (Hertog et al. 1995; Justesen et al. 1997; Kumpulainen et al. 1999). In the Seven Countries study, Japan had the highest intake of flavonoids followed by Croatia, whereas the Finnish cohort had lower intake (Hertog et al. 1995). DeVries and co-workers estimated the intake of five flavonoids in 17 different diets. Lowest intake (1 to 9 mg/day) was from South African diet, whereas highest flavonoid intake (75 to 81 mg/day) was from a Scandinavian diet (deVries et al. 1997). The reason of such very different intakes could be found in the difference of the nutritional habits, the composition of the diets, the flavonoid concentration of the plant foods, and others.

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