

FLAVONOID AGLYCONS IN FOODS OF PLANT ORIGIN II. FRESH AND DRIED FRUITS

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The content of potentially antioxidant, anticarcinogenic and antiallergic flavonoid aglycons, quercetin, kaempferol, myricetin, apigenin and luteolin of 45 fruits were determined by RP-HPLC with UV detection. Fresh and dried fruits were purchased in the local markets in Budapest at a period of their most frequent consumption. Total flavonoid content of fruits varied between 0–1000 mg kg⁻¹, the average concentration was about 30 mg kg⁻¹ fresh weight. Quercetin could be detected in most fruits, namely in apples, pear, plums, sweet and sour cherry and berries between 10–53 mg kg⁻¹. Luteolin at a concentration of 20 mg kg⁻¹ was found in melons, apples, kiwi and lemon. Myricetin was in detectable amount in redcurrant, and at very high concentration in some berry fruits (mulberry 453 mg kg⁻¹, raspberry 540 mg kg⁻¹, blackberry 636 mg kg⁻¹, strawberry 994 mg kg⁻¹), and in walnut (4565 mg kg⁻¹). Kaempferol and apigenin were not found in the fruits investigated. None of the five flavonoids was found in some variety of grapes, in peach, pear, banana, orange, grapefruit and tangerine, in nuts such as almond, pistachio, nuts, and in dried fruits such as raisin, date, fig and prunes. These data provide a basis for the evaluation of the average daily intake of Hungarian population and for an epidemiological evaluation of health-promoting effects of flavonoids.

Keywords: flavonoid aglycons, fresh fruits, dried fruits

Flavonoids are diphenylpropanes (C6-C3-C6) occurring ubiquitously in food plants and are common components in the human diet. Flavonoids are generally known to be present in plant flower, leaf, and fruit as pigments (KANDASWAMI & MIDDLETON, 1997). They are responsible for plant colours, i.e. the brilliant shade of blue, scarlet, orange, and other ones present in different plant's parts. They are found in various fruits, vegetables, nuts, seeds, grains, spices and herbs as well as in beverages such as tea, cocoa, beer and wine (KÜHNNAU, 1976) Although flavonoids generally are considered to be non-nutrient agents, interest in these substances has arisen because of their antioxidant and free radical scavenging abilities and potential effects on human health. Flavonoids, which constitute the most important group of polyphenols, can be divided into 13 classes such as flavonols, flavanones, flavanols, anthocyanidins, isoflavonoids, and others, with more than 5000 compounds described by 1990 (HARBORNE, 1994). Common structure of flavonoids consists of two aromatic rings linked through three carbons which usually

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form an oxygenated heterocycle. Figure 1 represents the basic structure and the system used for carbon numbering of the flavonoid nucleus. Biogenetically, the A ring usually comes from a molecule of resorcinol or phloroglucinol synthesized in the acetate pathway, whereas ring B is derived from the shikimate pathway (HARBORNE & MABRI, 1982). Flavonoids occasionally occur in plants as aglycones, although they are most commonly found as glycoside derivatives.

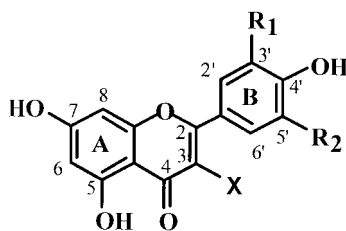


Fig. 1. Structure of flavonoids

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

Among the flavonoids, flavones (e.g. apigenin, luteolin, diosmetin), flavonols (e.g. quercetin, myricetin, kaempferol), and their glycosides are the most common compounds. They are widespread in the plant kingdom, with the exception of algae and fungi. The variability of these two groups is noteworthy, with about 380 flavonol glycosides and 200 different quercetin and kaempferol glycosides described to date (HARBORNE, 1994).

Flavonoids are known to display a bewildering array of pharmacological and biochemical actions recently reviewed by BRAVO (1998). They have been recognised as possessing anti-inflammatory, antiallergic, antiviral, antimicrobial, antihelminthic, hepatoprotective, antihormonal, antithrombotic, antimutagenic/anticarcinogenic, and antineoplastic activities. Flavonoids are very effective scavengers of hydroxyl and peroxy radicals, although their efficiency as scavengers of the superoxide anion is not totally clear (MANACH et al., 1996). They are chelators of transitional metal ions and inhibit the lipid peroxidation and the Fenton and Haber-Weiss reactions, which are important sources of active oxygen radicals (KANDASWAMI & MIDDLETON, 1997). Flavonoids are known to possess vitamin C stabilizing and antioxidant-dependent vitamin C-sparing activities (CLEMETSON & ANDERSEN, 1966). Epidemiological studies

have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of cardiovascular disease (HERTOG et al., 1993b, 1995, KATAN & HOLLMAN, 1998), and certain types of cancer (HERTOG, 1996).

Total flavonoid intake in the US was estimated to be around 1 g/day, of which about 100 mg (expressed as aglycones) consisted of flavonols and flavones (KÜHNAU, 1976). However these estimates were based on food composition data, which may have been inaccurate and incomplete. HERTOG and co-workers (1993b) calculated the intake of two types of flavonoids, flavonols and flavones in the Dutch diet, and found it to be 23 mg/day. More recently, LETH and JUSTESEN (1998) estimated the intake of flavones, flavonols, and flavanones in Denmark to be 28 mg/day. KUMPULAINEN and co-workers (1999) reported that the total average intake of 23 different flavonoid aglycons in Finland based on their food composition data was 55.2 mg/day.

On the basis of data from the literature (HERTOG et al., 1992b, 1993a, KUMPULAINEN et al., 1999) we selected three major flavonols, quercetin, kaempferol, and myricetin, and two major flavones, luteolin and apigenin. An HPLC method was adapted for the identification and quantification of these flavonoids in freeze-dried foods (HERTOG et al., 1992a). We have now reported the content of these flavonoids in 45 fresh and dried fruits commonly consumed in Hungary. The analytical data were compared to those published by HERTOG and co-workers (1992b) in The Netherlands and by LETH & JUSTESEN (1998) and JUSTESEN and co-workers (1998) in Denmark.

1. Materials and methods

1.1. Chemicals

Quercetin, luteolin, myricetin, kaempferol and t-butylhydroquinone were purchased from Sigma (St. Louis, MO, USA) apigenin from Fluka Chemie AG (Buchs, Switzerland) and methanol of chromatography grade were obtained from Merck KGaA (Darmstadt, Germany). All other chemicals and reagents were of analytical grade from Reanal (Hungary).

1.2. Fresh and dried fruits

Forty-five selected fresh and dried fruits (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 were used for the examination, and samples from three locations were combined. The skin of apples and pears was not removed. 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 105 °C. The food samples were stored at -18 °C for less than 4 months until analysed. Dried fruits and oily nuts were stored at 4 °C without lyophilization and they were chopped and homogenized immediately before the hydrolysis.

1.3. HPLC analysis

The flavonols (quercetin, kaempferol, myricetin) and the flavones (apigenin, luteolin) were measured as aglycons according to HERTOOG and co-workers (1992a). 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 it were 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 C₁₈ column (150 \times 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 $\mu\text{g ml}^{-1}$. Detector response was linear over the concentration range used. For all standards r^2 was higher than 0.998.

2. Results

From the results of HPLC analysis it basically became clear that fruits frequently consumed in Hungary did not contain kaempferol and apigenin at all. Although WILDANGER and HERRMANN (1973) reported kaempferol in some fruits such as cherry, plum, peach and redcurrant, the published quantities were very low (<10 mg kg^{-1} f.w.). HERTOOG and co-workers (1992b) also found a little amount of kaempferol in strawberry (12 mg kg^{-1}).

None of five measured flavonoids was detected in any varieties of grapes (Cardinal, Othello), green gooseberry, peach, quince-apple, pear, pomegranate, banana, grapefruit, orange, tangerine and poppy-seed, in oily nuts such as almond, pistachio, kashewnut, groundnut, hazel-nut and coco-nut, and in dried fruits such as raisin, date, fig, and prunes. HERTOOG and co-workers (1992b) published flavonoids in white and blue grapes at about 12–15 mg kg^{-1} for quercetin and less than 2 mg kg^{-1} for kaempferol. According to the literature, citrus fruits are richer sources of flavanones such as hesperidin, narirutin, eriocitrin and naringin than of flavones and flavonols analysed in our present study (TOMÁS-BARBERÁN & CLIFFORD, 2000). There are no data available on the flavonoid content of nuts. DANIEL and co-workers (1989) and JURD (1958) reported high level of ellagitannins in nuts (330–500 mg kg^{-1}). We supposed that polyphenols in nuts are predominantly tannins or other flavonoids not analysed in this study.

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

Table 1. Flavonoid content^a of stone fruits

Sample		Total flavonoids	Quercetin	Luteolin	Myricetin
Plum	Redskin	23.3	23.3	nd	nd
	Besztercei	12.3	12.3	nd	nd
Peach		nd	nd	nd	nd
Apricot		11.5	11.5	nd	nd
Greengage,	white skin	nd	nd	nd	nd
	red skin	nd	nd	nd	nd
Walnut		4565	nd	nd	4565

^a Mean (mg kg⁻¹ of fresh edible part) of duplicate determination
 nd: below the detection limit

concentration range of 11–23 mg kg⁻¹. Figures are presented in Table 1. Extremely high level of myricetin was found in walnut (4565 mg kg⁻¹), but other flavonoids were not present.

Berry fruits seem to be very rich sources of flavonoids, as it can be seen in Table 2. Quercetin was found in sweet and sour cherry (8.6 and 29.2 mg kg⁻¹), in gooseberry (9.1 mg kg⁻¹), in strawberry (9.7 mg kg⁻¹), in blackberry (14.5 mg kg⁻¹), in mulberry (24.7 mg kg⁻¹) and blackcurrant (52.8 mg kg⁻¹). Similarly to our results JUSTESEN and co-workers (1998) also found quercetin in blackcurrant at about 40 mg kg⁻¹. No quercetin was found in redcurrant although HERTOOG and co-workers (1992b) could detect it at about 10 mg kg⁻¹. They also reported quercetin in sweet cherry (15 mg kg⁻¹), in strawberry (9 mg kg⁻¹) and in white and blue grapes (12 and 15 mg kg⁻¹). Interestingly we found quercetin only in one grape variety namely Chasselas, but the concentration was three times higher than HERTOOG's data (38.7 mg kg⁻¹). HERTOOG and co-workers (1993a) also found high concentration of myricetin in grape juice (6.2 mg l⁻¹).

Extremely high concentration of myricetin was observed in some berries such as redcurrant, mulberry, raspberry, blackberry and strawberry (42.9, 452.5, 540.1, 636, and 993.6 mg kg⁻¹, respectively). HERTOOG and co-workers (1992b) did not find myricetin in these fruits at all. They detected myricetin only in blue grape at about 5 mg kg⁻¹. KUMPULAINEN and co-workers (1999) reported high level of flavonoids in berries: blackcurrant (quercetin 41 mg kg⁻¹, myricetin 53 mg kg⁻¹), cranberries (quercetin 104 mg kg⁻¹, myricetin 69 mg kg⁻¹), lingonberries (quercetin 100 mg kg⁻¹). Unfortunately they did not analyse strawberry, mulberry and blackberry, because mostly other berries are consumed in Finland. HÄKKINEN and co-workers (1999) found quercetin in strawberry and blackcurrant at a concentration of 6 and 53 mg kg⁻¹, respectively.

Table 2. Flavonoid content^a of berries

Sample	Total flavonoids	Quercetin	Luteolin	Myricetin
Sweet cherry	8.6	8.6	nd	nd
Sour cherry	29.2	29.2	nd	nd
Blackberry	650.5	14.5	nd	636
Raspberry	540.1	nd	nd	540.1
Strawberry	1003.3	9.7	nd	993.6
Blackcurrant	52.8	52.8	nd	nd
Redcurrant	42.9	nd	nd	42.9
Gooseberry				
green	nd	nd	nd	nd
red	9.1	9.1	nd	nd
Mulberry	477.3	24.7	nd	452.6
Grape				
Cardinal	nd	nd	nd	nd
Chasselas	38.7	38.7	nd	nd
Othello	nd	nd	nd	nd

^a Mean (mg kg⁻¹ of fresh edible part) of duplicate determination
nd: below the detection limit

Fruits similar to apple have luteolin and quercetin at a concentration around 20–30 mg kg⁻¹ (Table 3). Two varieties of apple contained quercetin (Gála 30.1 mg kg⁻¹, Golden 38.3 mg kg⁻¹) and two others luteolin (Golden 27.0 mg kg⁻¹, Jonatán 22.5 mg kg⁻¹). Similar concentrations were published by HERTOG and co-workers (1992b). They analysed six varieties of apple, but the differences among the cultivars were very low. In their study the mean quercetin content for all apple varieties was 36±19 mg kg⁻¹. They found that varieties purchased in December such as Elstar and Jonagold had the highest quercetin levels. They also found a little difference among three different pear varieties; the mean level was 6.4±3.4 mg kg⁻¹.

Table 3. Flavonoid content^a of apple-kind fruits

Sample	Total flavonoids	Quercetin	Luteolin	Myricetin
Apple				
Gála	30.1	30.1	nd	nd
Golden	65.3	38.3	27.0	nd
Jonathan	47.1	nd	22.5	nd
Pomegranate	nd	nd	nd	nd
Pear	nd	nd	nd	nd
Quince-apple	nd	nd	nd	nd

^a Mean (mg kg⁻¹ of fresh edible part) of duplicate determination
nd: below the detection limit

Table 4. Flavonoid content^a of melon-kind fruits

Sample	Total flavonoids	Quercetin	Luteolin	Myricetin
Water-melon	18.4	nd	18.4	nd
Muskmelon	25.8	nd	25.8	nd
Pumpkin	16.3	nd	16.3	nd

^a Mean (mg kg⁻¹ of fresh edible part) of duplicate determination
nd: below the detection limit

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⁻¹, respectively). There are no data available in the literature on the flavonoid content of these fruits. It is known that the dry material is very low in melons (<10%), thus the contribution of these fruits in the daily intake of flavonoids is supposed to be limited.

Flavonoid content of citrus and other exotic fruits are introduced in Table 5. Among citrus fruits only lemon has flavone luteolin at a concentration of 23.1 mg kg⁻¹ in the edible part. From other exotic fruits kiwi also contains luteolin (22.3 mg kg⁻¹). Although we did not find any flavonoids in grapefruit, tangerine and orange JUSTESEN and co-workers (1989) reported quercetin in freshly prepared juices from grapefruit and orange (4.9 and 5.7 mg l⁻¹, respectively). HERTOOG and co-workers (1993a) also found quercetin in juices of grapefruit, lemon and orange (4.9, 7.4 and 5.7 mg l⁻¹, respectively), and also myricetin in traces. Having these results authors expressed their surprise, because HERRMANN (1975) and BALESTIERI and co-workers (1991) reported that citrus flavonoids are almost exclusively flavanones.

Table 5. Flavonoid content^a of citrus and exotic fruits

Sample	Total flavonoids	Quercetin	Luteolin	Myricetin
Lemon	23.1	nd	23.1	nd
Grapefruit	nd	nd	nd	nd
Tangerine	nd	nd	nd	nd
Orange	nd	nd	nd	nd
Kiwi	22.3	nd	22.3	nd
Banana	nd	nd	nd	nd

^a Mean (mg kg⁻¹ of fresh edible part) of duplicate determination
nd: below the detection limit

3. Conclusion

Flavonoids are ubiquitous in foods of plant origin. However, accurate determination of dietary intakes is problematical owing to their immense diversity form and to variations in analytical methodology. In addition, their concentrations in foods can vary by many orders of magnitude and are influenced by several factors including species, variety, light, degree of ripeness, processing and storage (KÜHNNAU, 1976; HERRMANN, 1988; ROBARDS & ANTALOVICH, 1997; PETERSON & DWYER, 1998). Varietal differences in products also markedly confound the estimation of dietary intake. For example, quercetin contents of cherry tomatoes are approximately 30 mg kg⁻¹ compared with about 5 mg kg⁻¹ for those of normal size and the flavonol content of lettuce ranges from 10 to 900 mg kg⁻¹, depending on variety (CROZIER et al., 1997).

In our present study emphasis was placed on the identification and quantification of five major potentially antioxidant, anticarcinogenic, antiallergic and antimutagenic flavonoids in the edible parts of various commonly consumed fresh and dried fruits. Our investigation proves the presence of significant amount of different flavonoids in selected fruits frequently consumed in Hungary. In case of some fruits mainly due to their varietal differences our results are markedly different from those published by other authors. Together with data on the content of flavonoids in several vegetables already published (LUGASI & HÓVÁRI, 2000), a calculation of the daily intake of these potentially health-beneficial compounds can be made. On the basis of our results, the suggested increase of consumption of flavonoid-rich foods such as fresh fruits and vegetables is possibly a more appropriate strategy to increase intake of flavonoids than supplementation. Until we know more about the activity and metabolic fate of flavonoids in the body, it would be better to be very cautious about the consumption of supra-nutritional amounts of such bio-active compounds.

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