

# Quercetin, luteolin, apigenin and kaempferol contents of some foods

Sibel Karakaya\*, Sedef Nehir EL

Food Engineering Department, Engineering Faculty, Ege University, 35100 Bornova Izmir, Turkey

Received 7 October 1998; received in revised form and accepted 14 December 1998

## Abstract

Quercetin, luteolin, apigenin and kaempferol contents of *Urtica* sp., *Rosa canina* (rosehip), *Salvia officinalis* (sage), *Tilia platyphyllos* (linden flower), black tea, *Daucus carota* L. spp *sativus* (violet carrot juice), grape molasses, honey and tarhana were determined by HPLC with UV detection. Consumption of the samples was assessed using a food frequency questionnaire method. One hundred healthy volunteers were asked to state the number of times on average per day, week or month they consumed each item over the last 6 months. Quercetin was determined in all samples except honey, whereas luteolin was determined only in sage. Kaempferol was determined in black tea, linden flower and honey samples, and apigenin was determined in honey and *Urtica* sp. Quercetin intake by the consumption of tea, linden flower, sage rosehip, violet carrot juice, grape molasses, tarhana and juice of *Urtica* sp. were estimated as 4.2–25, 2.6, 3.3, and 2.0 µg/day, 1.67 mg/day, 1.70 mg/day, 1.78 mg/month and 21.75–65.25 µg/month, respectively. Luteolin intake by the consumption of sage was estimated as 1.32 µg/day. Apigenin intakes by the consumption of honey and juice of *Urtica* sp. were estimated as 2.34 mg/day and 3.58–10.73 mg/month, respectively. Kaempferol intakes of the participants from tea, linden flower and honey were estimated as 13.2–79.2, 13.56 and 190 µg/day respectively. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Flavonoids; *Urtica* sp.; Rosehip; Sage; Linden flower; Black tea; Violet carrot juice; Grape molasses; Honey; Tarhana

## 1. Introduction

Flavonoids occur naturally in plant foods and are a common component of our diet. They occur in foods generally as *O*-glycosides with sugar bound most often at the C-3 position. Flavonoids show a wide range of biochemical and antiallergic effects (Hertog, Feskens, Hollman, Katan & Kromhout, 1993b; Hertog, Hollman & Putte, 1993a; Hollman, Hertog & Katan, 1996). The results of in vitro studies have shown that flavonoids can inhibit, and sometimes induce, a large variety of mammalian enzyme systems. Some of these enzymes are involved in important pathways that regulate cell division and proliferation, platelet aggregation, detoxification, and inflammatory and immune response (Hollman et al., 1996).

The most important biological effect of flavonoids is their ability to act as antioxidants. Recently much attention has been paid to their antioxidant properties, which affect oxygen free radicals and lipid peroxidation. Oxygen free radicals and lipid peroxidation might be

involved in pathological conditions such as atherosclerosis, cancer and chronic inflammation (Briviba & Sies, 1994; Hollman et al., 1996; Husain, Cillard & Cillard, 1987; Jovanovic, Steenken, Hara & Simic, 1996; Robak & Gryglewski, 1988). Food-derived flavonoids, such as quercetin, kaempferol and myricetin, have antimutagenic and anticarcinogenic effects in vitro and in vivo (Hertog, Hollman & Katan, 1992a). Samejima, Kanazawa, Ashida and Danno (1995), reported that the desmutagenicity of luteolin was similar to that of galangin and stronger than that of quercetin. One possible desmutagenic mechanism of flavonoids is thought to be scavenging of the radicals before they damage the DNA. They also reported that flavonoids suppressed the mutagenicity of the other dietary carcinogens formed during the cooking process. It is well known that flavonoids can inhibit carcinogenesis by benzo (a) pyrene, dimethylbenz(a)anthracene, *N*-nitrosomethylurea and methylcholanthrene. Flavonoids in the diet are considered to be one of the most significant anticarcinogens.

Although a dietary guideline for the consumption of flavonoids has not yet been reported, there are many studies on the average intake of flavonoids. Hollman

\* Corresponding author.

et al. (1996) reported that the average intake of flavonols and flavones was 23 mg/day, of which the flavonol quercetin contributed 16 mg/day in The Netherlands. They indicated that this amount was in contrast with the data of Kühnau who estimated that the total intake of flavonoids in the United States was 1 g/day. The intake of flavonols and flavones was estimated to be, on average, 26 mg/day in the Zutphen study cohort, which was one of the cohorts of the Seven Countries Study (Hertog et al., 1993b). It was reported that the intake of flavonols and flavones ranged from 3 mg/day in a Finnish cohort to 70 mg/day in a Japanese cohort. The major dietary sources of flavonols and flavones varied substantially between cohorts. In the Japanese and Dutch cohorts the major source was tea, while red wine was the major source in Italy.

Although flavonoids generally are considered to be non-nutritive agents, interest in these substances has arisen because of possible effects on human health. In recent years, there have been many studies about the flavonoid content and antioxidative effects of green, semi-fermented and black teas (Gadow, Joubert & Hansmann, 1997; Hertog et al., 1993a; Kivits, van der Sman & Tjburg, 1997; Roeding-Penman & Gordon, 1997; Shao, Powell & Clifford, 1995; Vinson, Dabbagh, Serry & Jong, 1995). There are a few studies about the flavonoid content and antimutagenic activity of sage. Nataka et al. (1988) found that sage decreased the Trp-P-2 mutagenicity and Samejima et al. (1995) reported that the compound of sage responsible for this effect was luteolin. *Urtica* sp., rosehip, sage and linden flower have been especially used as herbal treatments for various health problems in Turkey. Also some of commonly consumed foods in Turkey are violet carrot juice, grape molasses, black tea, honey and tarhana.

The objectives of this study were to determine quercetin, luteolin, apigenin, and kaempferol contents of these herbal teas and other foods not recently studied.

## 2. Materials and methods

### 2.1. Source and preparation of the samples

All Turkish products except tarhana were purchased at various supermarkets of Izmir, Turkey. Brands selected were of commonly available in Turkey.

Tea, sage, linden flower, rosehip and *Urtica* sp.: four major brands of tea, two brands of sage, two brands of linden flower, which were harvested in summer, two kinds of rosehip and two kinds of *Urtica* sp., were each combined in equal portions to composites. Two hundred and fifty milliliters of boiling water were poured onto each 5 g of tea, sage, linden flower rosehip and *Urtica* sp. After 10 min, the infusions were passed through a sieve and allowed to cool prior to analysis.

Tarhana: two dry tarhanas from different origins (home) were used and combined in equal portions to a composite. Then 10 g of tarhana with 250 ml of water was cooked.

Violet carrot juice, grape molasses and honey: one violet carrot juice brand (1l) which is the most popular type was analysed. Two brands of grape molasses and two brands of honey were each combined in equal portions to composites prior to analysis.

### 2.2. Analysis

Four flavonoids, quercetin, luteolin, apigenin and kaempferol were determined in all samples after extraction and acid hydrolysis. In brief, 15 ml of tea, linden flower, rosehip, *Urtica* sp., tarhana and 10 ml of violet carrot juice, grape molasses or honey were taken. Ten milliliters of 6 M HCl and 40 ml aqueous methanol containing 2 g/l TBHQ were added to each extract of sample. After refluxing at 90°C for 2 h with regular swirling, the extracts were allowed to cool and volume subsequently made up to 100 ml with methanol. Then each sample was passed through a paper filter before chromatographic separations. Chromatographic separations were performed according to Hertog, Hollman and Venema (1992b) by HPLC on a  $\mu$ -Bondopak C<sub>18</sub> column using two mobile phases.

### 2.3. Food frequency questionnaires

Black tea, linden flower, sage, rosehip, violet carrot juice, grape molasses, tarhana, honey and *Urtica* sp. consumption of the 100 healthy adult volunteers was assessed using a food frequency questionnaire method. It consists of questions concerning the amount and consumption frequency of foods. The participants were asked to state the number of times on average, per day, week or month, they consumed each item over the last 6 months.

## 3. Results and discussion

Data were divided into two groups according to solid and liquid properties of the samples (Tables 1 and 3). Quercetin, luteolin, apigenin and kaempferol contents of black tea, linden flower, sage, rosehip, violet carrot juice and grape molasses are shown in Table 1. As seen in Table 1 all of the samples contain quercetin and only sage contains luteolin. The highest quercetin content was found to be in the grape molasses. Apigenin could not be determined in all samples.

Black tea is one of the most popular beverages in the world. Because of its popularity there are many studies about flavonoid contents of black tea. Hertog et al. (1993a) reported that quercetin was the most important

Table 1  
Flavonoids content of black tea, linden flower, sage, rosehip, violet carrot juice and grape molasses

	Quercetin ( $\mu\text{g/l}$ )	Luteolin ( $\mu\text{g/l}$ )	Apigenin ( $\mu\text{g/l}$ )	Kaempferol ( $\mu\text{g/l}$ )
Black tea	$34.8 \pm 9^a$	– <sup>b</sup>	–	$110 \pm 9$
Linden flower	$21.7 \pm 8$	–	–	$113 \pm 2$
Sage	$27.2 \pm 0.8$	$11 \pm 0.7$	–	–
Rosehip	$16.7 \pm 0.2$	–	–	–
Violet carrot juice	$83.7 \pm 0.5$	–	–	–
Grape molasses	$1692 \pm 28$	–	–	–

<sup>a</sup> Mean  $\pm$  standard deviation.

<sup>b</sup> Not detected.

flavonoid found in tea, with the exception of Jacksons Earl Grey tea and Lay's After dinner tea, in which kaempferol levels were higher. Our findings are in agreement with Hertog et al., 1993a. Kaempferol content of black tea and linden flower were found to be 110 and 113  $\mu\text{g/l}$ , respectively. Therefore kaempferol levels were higher than quercetin in these samples. Quercetin and kaempferol levels of various black tea were found to be 10–25 and 6.3–17  $\text{mg/l}$ , respectively, in their study. However, our results concerning the quercetin and kaempferol levels of black tea were lower than their results. These differences may be explained by the differences among the varieties, geographical origins, environmental conditions and fermentations which may affect the flavonoid composition of teas. Shahidi and Nacz (1995) indicated that flavonols, such as quercetin, myricetin and kaempferol, constitute 15 to 25% of the dry weight of tea. In another study, flavonoid content of black tea was found to be 32% (Gadow et al., 1997). In this study total flavonoid content of black tea was not studied. Determination of total phenolic content and antioxidant activity of black tea is in progress. Although there is increasing interest in the potential health effect of tea flavonoids, the bioavailabilities of these components are largely unknown. Hollman, Tijburg, and Yang (1997) reported that, in humans, the absorption of dietary quercetin, predominantly present as its glycoside in tea, ranged from 20 to 50%, depending on the presence and nature of the glycoside chain.

The highest number of the volunteers estimated from the questionnaire are given in Table 2. According to these consumption profiles, kaempferol and quercetin intake of the participants from tea were estimated as 13.2–79.2 and 4.2–25  $\mu\text{g/day}$ , respectively. Another beverage that contains quercetin and kaempferol is linden flower. The used form of linden flower is obtained after boiling of herbs and drinking the boiled juice as herbal tea, especially in winter. Linden flower is harvested in summer and in winter. Dried flowers of the herb are used for preparing herbal tea. Estimated intake of the kaempferol by the consumption of linden flower was found to be 13.56  $\mu\text{g/day}$ . Quercetin intakes by the

Table 2  
The highest number of volunteers estimated from food frequency questionnaires ( $n=100$ )

Foods	The number of volunteers
Tea (240–360 ml/day)	30
Linden flower (120 ml/day)	12
Sage (120 ml/day)	4
Rosehip (120 ml/day)	6
Grape molasses (10 ml/week)	24
Violet carrot juice (200 ml/month)	8
Tarhana (750 ml/month)	36
Honey (8 g/day)	28
Juice of <i>Urtica</i> sp. (2.5–7.5 g/month)	16

Table 3  
Flavonoid contents of tarhana, honey and *Urtica* sp.

	Quercetin ( $\text{mg}/100 \text{ g}$ )	Luteolin ( $\text{mg}/100 \text{ g}$ )	Apigenin ( $\text{mg}/100 \text{ g}$ )	Kaempferol ( $\text{mg}/100 \text{ g}$ )
Tarhana	$5.92 \pm 0.12^a$	–	–	–
Honey	– <sup>b</sup>	–	$29.3 \pm 4$	$2.42 \pm 0.1$
<i>Urtica</i> sp.	$0.87 \pm 0.02$	–	$143 \pm 55$	–

<sup>a</sup> Mean  $\pm$  standard deviation.

<sup>b</sup> Not detected.

consumption of linden flower, sage and rosehip were estimated as 2.6, 3.3 and 2.0  $\mu\text{g/day}$ , respectively. However, luteolin intake was estimated as 1.32  $\mu\text{g/day}$ .

Violet carrot juice is commonly consumed as a beverage in South Turkey. Violet carrot, which is produced locally in Hatay-Samandağ, is a subspecies of *Daucus carota* that contains violet pigment anthocyanins. Generally with violet carrot (*Daucus carota* L. spp. *sativus*), bulgur, which was traditionally made from durum wheat, salt, water and bread yeast is used as a raw material to prepare violet carrot juice. It has been produced by mixing the raw materials and allowing the mixture to continue lactic acid fermentation for several days (Canbas & Fenercioğlu, 1984; Özler & Kılıç, 1996). Quercetin was the only flavonoid found in violet carrot juice and grape molasses, and the quercetin intake of the participants, by the consumption of grape molasses and violet carrot juice, was estimated to be 1.70 and 1.67  $\text{mg/day}$ , respectively. Studies on the flavonoid content of grape molasses and violet carrot juice have not yet been reported.

Flavonoid contents of tarhana, honey and *Urtica* sp. are shown in Table 3. Quercetin was the major flavonoid found in tarhana whereas apigenin was the major flavonoid found in honey and *Urtica* sp.

Another flavonoid found in honey was kaempferol. Estimated intakes of apigenin and kaempferol from honey were 2.34 and 0.19  $\text{mg/day}$ , respectively. Ferreres, Tomas-Barberan, Gil and Tomas-Lorente (1991) reported that pinocembrin, pinobanksin, galangin, chrysin, luteolin, apigenin, isorhamnetin and quercetin-3-methyl ether

were the major compounds found in honey. Sabatier, Amiot, Tacchini and Aubert (1992) identified quercetin, pinobanksin, kaempferol, pinocembrin, chrysin, galangin and tectochrysin in sunflower honey. Andrade, Ferreres, Gil, and Tomas-Barberan (1997) reported that geographical and floral origin were the most important factors that affect flavonoid content of honey. We did not detect quercetin and luteolin in our honey samples. Differences among the flavonoid contents of the honey samples can be due to differences between their geographical and floral origins.

Fermented foods play an important role in the diets of many people in the Middle East. Tarhana is a traditional homemade food in Turkey. It has been prepared by mixing yoghurt, wheat flour, baker's yeast, onion, green pepper, fresh red pepper, peppermint, tomato and salt and allowing the mixture to continue lactic acid fermentation for several (5–7) days. The resulting material is air-dried and milled. Dry tarhana is cooked with water to consume as a soup, especially in winter. Quercetin intake of the participants from tarhana was estimated as 1.78 mg/month.

One of the herbs used frequently for traditional cancer treatment among Turkish people is *Urtica* sp. It is consumed by boiling of herbs and drinking the boiled juice. According to consuming pattern, estimated intake of apigenin was found to be 3.58–10.73 mg per month.

Studied samples play an important role in Turkish dietary patterns. Turkish people are used to consuming black tea at breakfast and after lunch. Also, violet carrot juice is usually taken with rakı, which is a traditional alcoholic beverage. Linden flower, sage and, rosehip are commonly used for treatment of various health problems. Therefore, further studies to investigate antioxidative effects of these samples are in progress.

## References

- Andrade, P., Ferreres, F., Gil, M. I., & Tomas-Barberan, F. A. (1997). Determination of phenolic compounds in honeys with different floral origin by capillary zone electrophoresis. *Food Chemistry*, 60 (1), 79–84.
- Briviba, K., & Sies, H. (1994). Nonenzymatic antioxidant defense systems. In B. Frei (Ed.), *Natural antioxidant in human health and disease* (pp. 107–128). New York: Academic Press.
- Canbas, A., & Fenercioğlu, H. (1984). Salgam suyu üzerinde bir araştırma. *Gıda*, 9 (5), 279–286.
- Ferreres, F., Tomas-Barberan, F. A., Gil, M. I., & Tomas-Lorente, F. (1991). An HPLC technique for flavonoid analysis in honey. *Journal of the Science Food Agriculture*, 56, 49–56.
- Gadow, A., Joubert, E., & Hansmann, C. F. (1997). Comparison of the antioxidant activity of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chemistry*, 60 (1), 73–77.
- Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992a). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *Journal of Agricultural Food Chemistry*, 40, 2379–2383.
- Hertog, M. G. L., Hollman, P. C. H., & Venema, D. P. (1992b). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural Food Chemistry*, 40, 1591–1598.
- Hertog, M. G. L., Hollman, P. C. H., & Putte, B. (1993a). Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. *Journal of Agricultural Food Chemistry*, 41, 1242–1246.
- Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., & Kromhout, D. (1993b). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, 342, 1007–1011.
- Hollman, P. C. H., Hertog, M. G. L., & Katan, M. B. (1996). Analysis and health effects of flavonoids. *Food Chemistry*, 57 (1), 43–46.
- Hollman, P. C. H., Tijburg, L.B.M., & Yang, C.S. (1997). Bioavailability of flavonoids from tea. *CRC Critical Reviews of Food Science and Nutrition*, 37 (8), 719–738.
- Husain, S. R., Cillard, J., & Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry*, 26 (9), 2489–2491.
- Jovanovic, S., Steenken, S., Hara, Y., & Simic, M.G. (1996). Reduction potentials of flavonoid and model phenoxyl radicals. Which ring in flavonoids is responsible for antioxidant activity? *J. Chem. Soc. Perkin Trans.*, 2, 2497–2504.
- Kivits, G. A. A., van der Sman, F. J. P., & Tijburg, L. B. M. (1997). Analysis of catechins from green and black tea in humans: a specific and sensitive colorimetric assay of total catechins in biological fluids. *Int. J. Food Sci. Nutr.*, 48, 387–392.
- Natake, M., Kanazawa, K., Mizuno, M., Ueno, N., Kobayashi, T., Danno, G., Robak, J., & Grylewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, 37 (5), 837–841.
- Özler, N., & Kılıç, O. (1996). Şalgam suyu üretimi üzerinde araştırmalar. *Gıda*, 21 (5), 323–330.
- Robak, J., & Grylewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemistry and Pharmacology*, 37(5), 837–841.
- Roeding-Penman, A., & Gordon, M. H. (1997). Antioxidant properties of catechins and green tea extracts in model food emulsions. *Journal of Agricultural Food Chemistry*, 45, 4267–4270.
- Sabatier, S., Amiot, M. J., Tacchini, M., & Aubert, S. (1992). Identification of flavonoids in sunflower honey. *Journal of Food Science*, 57 (3), 773–777.
- Samejima, K., Kanazawa, K., Ashida, H., & Danno, G. (1995). Luteolin: a strong antimutagen against dietary carcinogen, Trp-P-2, in peppermint, sage and thyme. *Journal of Agricultural and Food Chemistry*, 43, 410–414.
- Shahidi, F., & Naczk, M., 1995. Food phenolics sources chemistry effects applications (pp. 109–158). Lancaster/Basel: Technomic.
- Shao, W., Powell, C., & Clifford, M. N. (1995). The analysis by HPLC of green, black and Pu'er teas produced in Yunnan. *Journal of the Science of Food Agriculture*, 69, 535–540.
- Vinson, J. A., Dabbagh, Y. A., Serry, M. M., & Jong, J. (1995). Plant flavonoids especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. *Journal of Agricultural Food Chemistry*, 43, 2800–2802.