Content of Potentially Anticarcinogenic Flavonoids of Tea Infusions, Wines, and Fruit Juices†

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The content of the potentially anticarcinogenic flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin of commonly consumed beverages was determined by RP-HPLC with UV detection. Flavonoid levels in beer, coffee, chocolate milk, and white wine were below 1 mg/L. Twelve types of tea infusion, six types of wine, apple juice, tomato juice, grape juice, orange juice, grapefruit juice, and lemon juice were analyzed. No luteolin or apigenin were detected in any of the beverages. In red wines and in grape juice quercetin and myricetin levels varying from 4 to 16 mg/L and from 7 to 9 mg/L, respectively, were detected. Quercetin levels in fruit juices were generally below 5 mg/L except for lemon juice (7 mg/L) and tomato juice (13 mg/L). In black tea infusions quercetin (10–25 mg/L), kaempferol (7–17 mg/L), and myricetin (2–5 mg/L) were detected. Flavonoid levels in green tea were comparable to those in black tea. The flavonoid content of tea prepared with tea bags was generally higher than that of tea prepared with loose leaves. Together with data on the flavonoid content of vegetables and fruits published previously (Hertog et al. *J. Agric. Food Chem.* **1992**, *40*, 2379–2383), these data provide a base for an epidemiological evaluation of the potentially anticarcinogenic effects of flavonoids.

INTRODUCTION

Flavonoids occur naturally in plant foods and are a common component of our diet. They generally occur as *O*-glycosides with sugars bound at the C3 position. Flavonoids demonstrated a wide range of biochemical and pharmacological effects, including antiinflammatory and antiallergic effects (Middleton and Kandaswami, 1992). Food-derived flavonoids such as quercetin, kaempferol, and myricetin (Figure 1) also inhibited carcinogen-induced tumors in rats and in mice (Mukhtar et al., 1988; Verma et al., 1988; Wei et al., 1990; Deschner et al., 1991). In contrast, quercetin was also found to induce bladder tumors in rats at a level of 2% in the diet (Pamucku et al., 1980). However, these results could not be confirmed in other animal studies using quercetin levels of up to 10% of the diet (Saito et al., 1980; Hirono et al., 1981; Morino et al., 1982). Antioxidant flavonoids such as quercetin inhibited oxidation and cytotoxicity of low-density lipoproteins in vitro (De Whalley et al., 1990; Negre-Salvagyre and Salvagyre, 1992), which may decrease their atherogenicity and subsequent risk for coronary heart disease (Steinberg et al., 1989). An epidemiological evaluation of the effects of flavonoids on chronic diseases such as cancer and cardiovascular disease is needed to support the findings from experimental studies.

Flavonoids consist mainly of anthocyanidins, flavonols, flavones, catechins, and flavanones (Herrmann, 1988). Important dietary sources of flavonoids are vegetables, fruits, and beverages, the latter accounting for at least 25–30% of the total daily flavonoid intake (Kühnau, 1976). Kühnau estimated that the intake of all flavonoids in the United States is approximately 1 g/day. However, this estimation was based mainly on food analyses using techniques of doubtful accuracy. We developed a HPLC method for the determination of three major flavonols, quercetin, kaempferol, and myricetin, and two major flavones, luteolin and apigenin, in foods (Hertog et al., 1992a). These flavonoids were selected because they are most widely investigated in anticarcinogenesis studies. In a previous study we reported the flavonoid content of 28 vegetables and 9 fruits (Hertog et al., 1992b). Here, we report the flavonoid content of 12 types of tea, 6 types of wine, and 7 types of fruit juice.

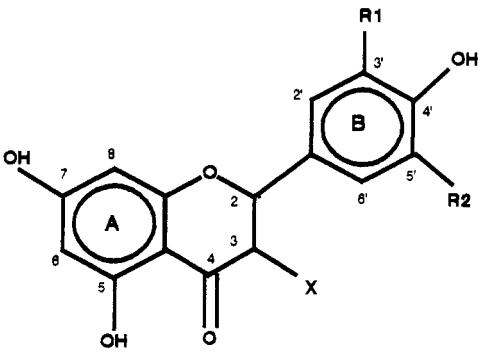

Figure 1. Structure of flavonoids: flavonols, X = OH; quercetin, R_1 = OH, R_2 = H; kaempferol, R_1 = H, R_2 = H; myricetin, R_1 = OH, R_2 = OH; flavones, X = H; apigenin, R_1 = H, R_2 = H; luteolin, R_1 = OH, R_2 = H.

MATERIALS AND METHODS

Sources and Preparation of Beverages. All Dutch products were purchased in an outlet of a nationwide supermarket chain (Albert Heijn) in the period February–August 1992. Products and brands were selected on the basis of information obtained from the Dutch Commodity Board of Distillates and the Dutch Commodity Board of Tea and Coffee. We included typical types of English tea that are not commonly available in The Netherlands (Lay's English Breakfast, Jacksons Earl Grey, St. Michael Extra Strong, and Lay's After Dinner) which were kindly provided by Dr. S. Bingham (Cambridge, U.K.). Although green tea is not commonly consumed in The Netherlands, we included it in our study because several reports on the cancer-protective effects of

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green tea have been published (Conney et al., 1992; Katiyar et al., 1992; Stich, 1992).

Wine. All wines were averagely priced wines commonly consumed in The Netherlands. Three bottles (750 mL) of red French Bordeaux wines from different regions (Grand vin Bordeaux app. Lussac St. Emilion contr. 1990, App. Bordeaux contr. Rineaux St. Loubes 1990, and App. Superieur Bordeaux contr. Rineaux St. Loubes 1990) were purchased and combined in equal portions to a composite. Three white German wines from different origins (Mosel-Saar Ruwer Graacher Himmelreich Riesling 1991, Mosel-Saar Ruwer Bernkasteler Kurfürst Lay 1990, and Rheinpfalz Scheigener Guttenberg Kabinett 1989) were mixed in equal portions to a composite. Furthermore, one red Italian Chianti wine (1990), one red Spanish Rioja wine (Otonal 1990), one red Californian wine (Dry Pinot Noir 1990), and one white French Bordeaux wine (St. Loubes Rineaux 1990) were purchased.

Tea. Twelve types of tea (*Camellia sinensis* L.), including black, green, and oolong tea, were prepared following the manufacturer's guidelines. Preparation was, unless otherwise indicated, as follows: Five hundred milliliters of boiling water was poured onto 5 g of loose tea leaves. After 5 min, the infusion was passed through a sieve and allowed to cool prior to analysis. Tea in bags containing 4.0 or 5.0 g was placed during 5 min in 500 mL of boiling water. The tea bag was then removed, and the liquid was allowed to cool prior to analysis. The influence of brewing time on flavonoid yield was studied by varying the time before the infusion (Lay's After Dinner tea) was passed through a sieve (5, 10, and 20 min). We also studied the effect of the amount of tea used for brewing on flavonoid yield (4.0, 5.0, and 6.0 g) using Lay's English Breakfast tea.

Flavonoid levels of infusions prepared with tea bags proved to be consistently higher than infusions prepared with loose tea leaves. We noted that the particle size of tea in tea bags (approximately 0.4–0.8 mm) was much smaller than that of loose tea leaves. To study the effect of particle size on flavonoid yield, loose tea leaves (Lays's English Breakfast tea) were ground and the fraction with a particle size between 0.4 and 0.8 mm was collected. We then compared flavonoid levels in tea infusions, prepared with untreated loose leaves, with those found in tea infusions prepared with the 0.4–0.8-mm fraction of the same tea leaves obtained after grinding.

Coffee, Chocolate Milk, and Beer. Three bottles of Heineken beer (0.33 cL) were purchased and combined into a composite. One liter of commonly consumed chocolate milk (Albert Heijn's own brand) made of semiskimmed milk was purchased. The coffee (Zilvermerk, Douwe Egberts, Utrecht, The Netherlands) was similar to the most popular types of coffee sold in The Netherlands. Coffee was made by pouring 125 mL of boiling water on 4.0 g of coffee, which then dripped through a paper filter into a cup.

Fruit Juice. Two major brands of tomato juice (1 L each) (Albert Heijn's own brand, Zaandam, The Netherlands, and Zontomaatje, Riedel, Ede, The Netherlands) and two orange juice brands (1 L each) (Albert Heijn's own brand and Appelsientje, Riedel) were each combined in equal portions to composites prior to analysis. One apple juice brand (Goudappeltje, Riedel) and one grape juice brand (Albert Heijn's own brand) (1 L each) were analyzed. All fruit juices consisted of 100% fruit, as indicated by the producer. Fresh orange juice, fresh lemon juice, and fresh grapefruit juice were each made by squeezing 1 kg of fresh oranges (*Citrus sinensis* L.), 1 kg of fresh lemons (*Citrus medica* L.), and 1 kg of fresh grapefruits (*Citrus maxima* Merr.) with a common household fruit squeezer.

Methods of Analysis. Five flavonoids, viz. quercetin, kaempferol, myricetin, luteolin, and apigenin, were determined in beverages after extraction and acid hydrolysis of the flavonoid glycosides. This method of analysis originally was developed for the determination of flavonoids in freeze-dried foods (Hertog et al., 1992a) and was therefore slightly adapted for analyses of beverages. In brief, 15 mL of sample was boiled with HCl in 50% aqueous methanol, leading to hydrolysis of the flavonoid glycosides to their aglycons and simultaneous extraction of the aglycons. The resulting aglycons were quantified by RP-HPLC on a Nova-Pak C_{18} column using acetonitrile/phosphate buffer (25/75 v/v, pH 2.4) as mobile phase and UV detection (370 nm).

Table I. Results of Analytical Quality Control Sample[a]

	control sample[b]					
compound	mean[c] at start	SD_R	CV_R, %	mean[c] series	SD_R	CV_R, %
quercetin	1141 (n = 4)	60	5.3	1121 (n = 7)	36	3.2
myricetin	572 (n = 4)	30	5.2	590 (n = 7)	27	4.6

[a] Each determination was carried out duplicate. [b] Freeze-dried cranberries. [c] mg/kg dry weight, within-laboratory standard deviation of reproducibility (SD_R), and within-laboratory coefficient of variation of reproducibility (CV_R) at start and in the whole project (0.5 year).

To confirm peak identity, sample extracts were reinjected, using methanol/phosphate buffer (45/65 v/v, pH 2.4) as mobile phase. In addition, peak identity and purity were confirmed using a photodiode array detector to record UV spectra of the flavonoids in samples on-line. All determinations were carried out in duplicate. Limit of detection was defined as the amount of flavonoids resulting in a peak height of 3 times the standard deviation of the baseline noise.

As completeness of hydrolysis largely depends on the type of glycosides, we determined in a preliminary experiment optimum hydrolysis conditions in beverages as described elsewhere (Hertog et al., 1992b). Briefly, three HCl concentrations (1.2, 1.6, and 2.0 M) and hydrolysis periods of 2 and 4 h at 90 °C were tested. Optimum conditions were as follows: wines were hydrolyzed with 1.2 M HCl during 4 h at 90 °C; other beverages were hydrolyzed with 1.2 M HCl during 2 h at 90 °C (results not shown).

Analytical Quality Control. Precision of the method of analysis was reported before (Hertog et al., 1992a) for freeze-dried products. For beverages the following analytical variation was measured. Coefficients of variation of repeatability (CV_R) of quercetin in tea (n = 17), wines (n = 4), and fruit juices (n = 7) were 1.9, 4.5, and 4.5%, respectively. CV_R of kaempferol in tea (n = 17) was 3.8%, whereas the CV_R of myricetin in tea (n = 17) was 4.6% and in wine (n = 4), 2.4%. Recoveries were determined in duplicate in black tea (melange, Douwe Egberts) by spiking pure standards to the extraction solution (100% of the measured content) prior to sample analysis. Mean recoveries were 86.4% for quercetin and 93.2% for kaempferol.

One control sample, consisting of freeze-dried cranberries, was included in each series of samples. At the start of the project, which took about half a year, the quercetin and myricetin contents were determined by four duplicate analyses on different days within a period of 4 weeks to determine within-laboratory standard deviation of reproducibility (SD_R). Each series of analyses (n = 7) consisted of the control sample and three calibration samples placed at the beginning and ending of the series. All determinations were carried out in duplicate. Differences between duplicates of more than 10% were not accepted. Series of analyses were repeated whenever the control sample exceeded the confidence limits (mean ± 3 SD_R). Accepted results of the control sample are shown in Table I. Regression analysis of the results of the control sample did not reveal an association between flavonoid content and time of analysis. Long-term variability of flavonoid analyses in the laboratory was low (CV_R series <5%).

RESULTS

Table II reports the quercetin and myricetin content of wine and fruit juice. No apigenin, luteolin, or kaempferol could be detected in any of these beverages (limit of detection 0.5 mg/L). In general, myricetin was the most important flavonoid found in wines and grape juice, with the exception of the red Chianti and the red Californian wine in which quercetin predominated. In white wines we did not detect any quercetin or myricetin, except for the white German Mosel composite in which a very low level of myricetin (1 mg/L) was found. Quercetin levels varied among different types (4–16 mg/L), whereas the myricetin levels were similar in all red wines (7–9 mg/L). Highest flavonoid levels were found in the Italian Chianti wine. The quercetin and myricetin content of grape juice

Table II. Quercetin and Myricetin Contents of Wine, Fruit Juices, and Other Beverages

beverage	mg/L[a] quercetin	myricetin
wine		
red Bordeaux (composite)[b]	4.1	7.5
red Rioja Otonal 1990	4.1	9.3
red Chianti 1990	16	8.0
red California Dry Pinot Noir 1990	8.8	6.9
white Bordeaux St. Loubes Rineaux 1990	<0.5	<0.5
white Mosel (composite)[c]	<0.5	1.0
fruit juice		
apple juice (Albert Heijn, Zaandam)	2.5	<0.5
grape juice (Riedel, Ede)	4.4	6.2
tomato juice (commercial composite)[b]	13	<0.5
grapefruit juice (fresh)	4.9	<0.5
lemon juice (fresh)	7.4	<0.5
orange juice (fresh)	3.4	<0.5
orange juice (commercial composite)[e]	5.7	<0.5
other beverages		
beer (Heineken)	<0.5	<0.5
chocolate milk (semiskimmed milk)	1.3	<0.5
coffee	<0.5	<0.5

[a] Mean of duplicate determination; <0.5 below the limit of detection. [b] 1, Grand vin Bordeaux app. Lussac St Emillion contr. 1990; 2, App. Bordeaux contr. Rineaux St. Loubes 1990; 3, App. Superieur Bordeaux contr. Rineaux St. Loubes 1990. [c] 1, Mosel-Saar Ruwer, Graacher Himmelreich Riesling, 1991; 2, Mosel-Saar Ruwer, Bernkasteler Kurfürst Lay, 1990; 3, Rheinpfalz Scheigener Guttenberg Kabinett 1989. [d] Albert Heijn's own brand and Zontomaatje (1:1 v/v). [e] Albert Heijn's own brand and Appelsientje (1:1 v/v).

Table III. Contents of Quercetin, Kaempferol, and Myricetin in Different Types of Tea Infusions

tea[a]	amount (b = teabag)	mg/L[b] quercetin	kaempferol	myricetin
Pickwick DE melange[c] black	4.0 g (b)	19	15	3.2
Van Nelle melange[c] black	4.0 g (b)	17	14	2.8
Albert Heijn melange[c] black	4.0 g (b)	17	13	3.0
St. Michael extra strong black	5.0 g (b)	21	15	2.5
Lipton "Brisk" black	5.0 g (b)	25	16	5.2
Pickwick DE Earl Grey black	4.0 g (b)	21	17	3.5
Jacksons Earl Grey black	5.0 g	12	16	2.5
Lay's After Dinner black	5.0 g	10	12	2.5
brewing time 10 min	5.0 g	12	14	3.0
brewing time 20 min	5.0 g	13	14	2.5
Lay's English Breakfast black	4.0 g	10	6.3	1.7
	5.0 g	11	7.0	2.1
	6.0 g	16	9.5	3.1
ground fraction (0.4–0.8 mm)	5.0 g	16	9.2	2.9
Formosa, oolong	5.0 g	13	9.0	4.9
Japan, "Sencha" green	5.0 g	23	15	12
China, "Gunpowder" green	5.0 g	14	9.1	5.2

[a] Brewing time was 5 min unless otherwise indicated. [b] Mean of duplicate determinations. [c] Mixture of English Breakfast and Afternoon tea.

compared well with the levels found in red wines. In other fruit juices only quercetin was found, with the highest amounts found in tomato juice (13 mg/L). The quercetin level of fresh orange juice compared well with the quercetin level in industrially produced orange juice (fruit content 100%). No flavonoids were found in beer or in brewed coffee. In chocolate milk we found a very low amount of quercetin (1 mg/L).

The quercetin, kaempferol, and myricetin contents of various types of teas are shown in Table III. A typical chromatogram of a black tea extract is shown in Figure 2. No apigenin or luteolin could be detected in tea. Quercetin was in general the most important flavonoid found in tea, with the exception of Jacksons Earl Grey tea and Lay's After Dinner tea, in which kaempferol levels were higher. Black tea infusions prepared with tea bags (4.0 or 5.0 g) contained 17–25 mg/L quercetin, 13–17 mg/L kaempferol,

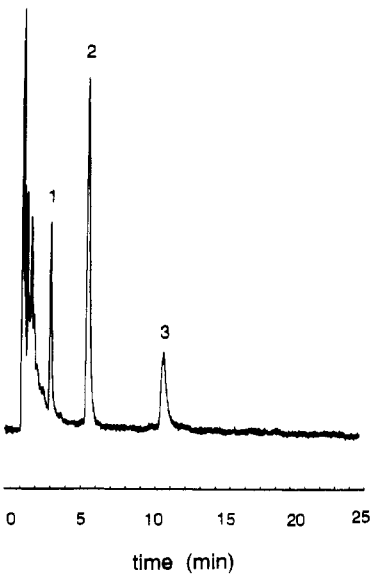

Figure 2. Typical chromatogram of a black tea infusion (Lipton "Brisk") extract monitored in 25% acetonitrile/phosphate buffer (pH 2.4). Peaks: myricetin (1), quercetin (2), and kaempferol (3). Detection was at 370 nm; 0.01 AUFS; flow rate, 0.9 mL/min.

and approximately 3 mg/L myricetin. The quercetin content of black tea infusions prepared with loose leaves was considerably lower (10–13 mg/L). Flavonoid yield in black tea (Lay's After Dinner) was slightly higher when the brewing time was extended to 10 min, but it did not increase after 10 min. There was also an increase in flavonoid yield with increasing amount used for brewing, but this increase was not linear. Grinding of the tea leaves had a pronounced effect on flavonoid yield. Quercetin levels increased by approximately 40% from 11 mg/L in untreated tea to 16 mg/L in tea prepared with the 0.4–0.8-mm fraction of the ground leaves. However, this increase was less pronounced for kaempferol and myricetin. Flavonoid levels in oolong tea were generally in the lower range (5–13 mg/L) of the flavonoid content of black tea. The amounts of flavonoids found in both green tea infusions are comparable to the average levels found in black tea, except for myricetin, which was higher (5–12 mg/L) than in black tea (2–5 mg/L).

DISCUSSION

Most studies on the flavonoid content of beverages such as wine, fruit juices, and tea have been carried out for taxonomic purposes. This implies that in general only the major flavonoid glycosides, or in fermented products such as tea and wine the major free flavonoid aglycons, were identified. Only little attention has been paid to the quantitative determination of flavonoids in beverages after hydrolysis of the glycosides to their corresponding aglycons. A quantitative comparison between our values and those reported in the literature is therefore difficult. The large variation in flavonoid content of different types of red wines is confirmed by Salagoïty-Auguste and Bertrand (1984), Revilla et al. (1986), and Etievant et al. (1988). We could not detect flavonoids in white wines, which is consistent with Revilla et al. (1986), who reported quercetin levels in white Spanish wines below our limit of detection.

Unexpectedly, we detected quercetin in fresh orange, grapefruit, and lemon juices and in commercial orange juice. Herrmann (1976) and Balestieri et al. (1991) reported that citrus fruits contain almost exclusively flavanones. Flavanone analyses in fruit juices have been mainly carried out for tracking down adulterations. The

applied analytical techniques may thus have lacked sensitivity for detection of quercetin. To our knowledge, no data on the flavonoid content of tomato juice, apple juice, and grape juice have been published previously. Quercetin was also found in fresh tomatoes, apples, and grapes (Hertog et al., 1992b). We could not detect any of the investigated flavonoids in beer or coffee, and only a very low amount was detected in chocolate milk. Kühnau estimated in 1976 that these beverages were dietary sources of flavonoids, but this could not be confirmed in our study.

Flavonoid levels in different types of black tea varied only slightly. The size of tea levels and consequently extraction surface seemed to be of far more importance. Particle size thus explains largely the differences in flavonoid yield between tea prepared with tea bags and tea prepared with loose leaves. A brewing time of tea of 20 min, as is customary in some countries such as the United Kingdom, did not result in an important increase in flavonoid yield. An ordinary brewed tea infusion (made by pouring 500 mL of boiling water on 5 g of tea leaves and a brewing time of 5 min) thus contains 30–40 mg/L of combined flavonoids and contributes therefore significantly to flavonoid intake in humans. Green tea infusions contained a similar amount of flavonoids as black tea. It should be noted that green tea used in this study had large-size leaves compared to the loose black tea. The total content of combined flavonoids in Japanese green tea "Sencha" amounts to 50 mg/L.

In only one study the flavonoid content of tea infusion was reported. Fieschi et al. (1989) determined the total flavonoid glycoside and aglycon contents of different types of black tea infusions using paper chromatography followed by spectrophotometric measurements. Total flavonoid content varied from 46 to 86 mg/L and the content of flavonoid aglycons from 0.8 to 1.1 mg/L. Total flavonoid content of green tea was 82 mg/L and aglycon content 2 mg/L. In most other studies loose tea leaves were extracted and analyzed directly for flavonoid glycosides. Biedrich et al. (1989) reported 860–2140 mg/kg rutin (quercetin 3-*O*-rhamnoglucoside) determined by HPLC in different types of black tea. Baily et al. (1990) and Finger and Engelhardt (1991) reported that the contents of quercetin and kaempferol rhamnodiglucosides determined by HPLC in different types of black tea were 410–950 and 500–1200 mg/kg, respectively. These figures are generally lower than our values. We found approximately 2400–2875 mg/kg quercetin and 1625–2125 mg/kg kaempferol extracted in the black tea infusion.

In conclusion, the data of the present study and the results of the previous study (Hertog et al., 1992b) provide a base for epidemiological studies investigating the relation between the intake of these antioxidant flavonoids and the risk of chronic diseases such as cancer and coronary heart disease.

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LITERATURE CITED

Baily, R. G.; McDowell, I.; Nursten, H. E. Use of an HPLC photodiode array detector in a study of the nature of black tea liquor. *J. Sci. Food Agric.* **1990**, *52*, 509–525.

Balestieri, F.; Flori, A.; Marini, D. Strategies for food quality control and analytical methods in Europe. Proceedings of the 6th European Conference on Food Chemistry. *Lebensmittelchem. Gesellschaft* **1991**, *23*, 369–378.

Biedrich, P.; Engelhardt, U. H.; Herzig, B. Determination of quercetine-3-*O*-rutinoside in black tea by HPLC. *Z. Lebensm. Unters. Forsch.* **1989**, *189*, 149–150.

Conney, A. H.; Wang, Z.-Y.; Huang, M.-T.; Ho, C.-T.; Yang, C. S. Inhibitory effect of green tea on tumorigenesis by chemicals and ultraviolet light. *Prev. Med.* **1992**, *21*, 361–369.

Deschner, E. E.; Ruperto, J.; Wong, G.; Newmark, H. L. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **1991**, *7*, 1193–1196.

De Whalley, C. V.; Rankin, S. M.; Hoult, J. R. S.; Jessup, W.; Leake, D. S. Flavonoids inhibit the oxidative modification of low density lipoproteins by macrophages. *Biochem. Pharmacol.* **1990**, *39*, 1743–1750.

Etievant, P.; Slich, P.; Bertrand, A.; Symonds, P.; Bouvier, J-C. Varietal and geographic classification of french red wines in terms of pigments and flavonoid compounds. *J. Sci. Food Agric.* **1988**, *42*, 39–54.

Fieschi, M.; Codignola, A.; Luppi Mosca, A. M. Mutagenic flavonol aglycones in infusions and in fresh and pickled vegetables. *J. Food Sci.* **1989**, *42*, 1492–1495.

Finger, A.; Engelhardt, U. H. Flavonol glycosides in tea – kaempferol and quercetin rhamnodiglucosides. *J. Sci. Food Agric.* **1991**, *55*, 313–321.

Herrmann, K. Flavonols and flavones in food plants. A review. *J. Food Technol.* **1976**, *11*, 433–448.

Herrmann, K. On the occurrence of flavonol and flavone glycosides in vegetables. *Z. Lebensm. Unters. Forsch.* **1988**, *186*, 1–5.

Hertog, M. G. L.; Hollman, P. C. H.; Venema, D. P. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *J. Agric. Food Chem.* **1992a**, *40*, 1591–1598.

Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. The content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J. Agric. Food Chem.* **1992b**, *40*, 2379–2383.

Hirono, I.; Ueno, I.; Hosaka, S.; Takanashi, H.; Matsushima, T.; Sugimura, T.; Natori, S. Carcinogenicity examination of quercetin and rutin in ACI rats. *Cancer Lett.* **1981**, *13*, 15–21.

Katiyar, S. K.; Agarwal, R.; Wang, Z. Y.; Bhatia, A. K.; Mukhtar, H. (–)-Epigallocatechin-3-gallate in camellia sinensis leaves from Himalayan Region of Sikkim: Inhibitory effects against biochemical events and tumor initiation in SENCAR mouse skin. *Nutr. Cancer* **1992**, *18*, 73–83.

Kühnau, J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* **1976**, *24*, 117–120.

Middleton, E.; Kandaswami, C. Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharmacol.* **1992**, *43*, 1167–1179.

Morino, K.; Matsukura, N.; Ohgaki, H.; Kawachi, T.; Sugimura, T.; Hirono, I. Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration. *Carcinogenesis* **1982**, *3*, 93–97.

Mukhtar, H.; Das, M.; Khan, W. A.; Wang, Z. Y.; Bik, D. P.; Bickers, D. R. Exceptional activity of tannic acid among naturally occurring plant phenols in protecting against 7,12-dimethylbenz(a) anthracene-, benzo(a)pyrene-, 3-methylcholanthrene-, and N-methyl-N-nitrosourea-induced skin tumorgenesis in mice. *Cancer Res.* **1988**, *48*, 2361–2365.

Negre-Salvagyre, A.; Salvagyre, R. Quercetin prevents the cytotoxicity of oxidized low density lipoproteins by macrophages. *Free Radical Biol. Med.* **1992**, *12*, 101–106.

Pamucku, A. M.; Yalçiner, S.; Hatcher, J. F.; Bryan, G. T. Quercetin, a rat intestinal and bladder carcinogen present in bracken fern (*Pteridium aquilinium*). *Cancer Res.* **1980**, *40*, 3468–3472.

Revilla, E.; Alonso, E.; Estrella, M. I. Analysis of flavonoid aglycones in wine extracts by High Performance Liquid Chromatography. *Chromatographia* **1986**, *22*, 1–6.

Saito, D.; Shirai, A.; Matsushima, T.; Sugimura, T.; Hirono, I. Test of carcinogenicity of quercetin, a widely distributed mutagen in food. *Teratog., Carcinog. Mutagen.* **1980**, *1*, 213–221.

Salagoïty-Auguste, M.-H.; Bertrand, A. Wine phenolics—analysis of low molecular weight components by High Performance Liquid Chromatography. *J. Sci. Food Agric.* **1984**, *38*, 1241–1247.

Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Modifications of low-density lipoprotein that increase its atherogenicity. *N. Engl. J. Med.* **1989**, *320*, 915–924.

Stich, H. F. Teas and tea components as inhibitors of carcinogen formation in model systems and man. *Prev. Med.* **1992**, *21*, 377–384.

Verma, A. K.; Johnson, J. A.; Gould, M. N.; Tanner, M. A. Inhibition of 7,12-dimethylbenz(a)anthracene and N-nitrosomethylurea induced rat mammary cancer by dietary flavonol quercetin. *Cancer Res.* **1988**, *48*, 5754–5788.

Wei, H.; Tye, L.; Bresnick, E.; Birt, D. F. Inhibitory effect of apigenin, a plant flavonoid, on epidermal ornithine decarboxylase and skin tumor promotion in mice. *Cancer Res.* **1990**, *50*, 499–502.

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