

## Flavonoids in Vegetable Foods Commonly Consumed in Brazil and Estimated Ingestion by the Brazilian Population

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The objective of this work was to quantify the flavonoids present in foods most commonly consumed by the Brazilian population. The predominant flavonoids found in largest abundance in all of the analyzed vegetables were glycosides of quercetin. In lettuce, a small amount of luteolin was also detected. In sweet pepper, quercetin and luteolin were both present. White onion [48–56 mg/100 g of fresh weight (FW), expressed as aglycon], red onion (40–100 mg/100 g of FW), red lettuce (67–67.2 mg/100 g of FW), arugula (41–118 mg/100 g of FW), and chicory (18–38 mg/100 g of FW) were highest in total flavonoids. In fruits, the highest concentrations of flavonoids were found in the peel (125–170 mg/100 g of FW) and pulp (35–44 mg/100 g of FW) of oranges and in some apple varieties (14–36 mg/100 g of FW). Variability in flavonoid content due to time of harvesting was high for leafy vegetables and red onions. The estimated ingestion by Brazilian population ranged from 60 to 106 mg/day.

**KEYWORDS:** Brazilian vegetable foods; flavonoids; time of harvesting, daily intake

### INTRODUCTION

Several epidemiological studies suggest that vegetables and fruits have a protective effect against cancer (1, 2). An exciting hypothesis is that vegetables may contain compounds that have beneficial effects independent of well-known nutrients and micronutrients. Researchers have demonstrated a wide range of biochemical and pharmacological effects, including anti-inflammatory and antioxidant actions, for a class of phytochemicals called flavonoids (3–5).

Flavonoids are diphenylpropanes (C<sub>6</sub>–C<sub>3</sub>–C<sub>6</sub>) ubiquitous in plants and are an integral part of the human diet. They occur in foods generally as *O*-glycosides (6, 7) and are classified in flavonols, flavones, flavanones, catechins (flavanols), anthocyanidins, isoflavones, dihydroflavonols, and chalcones, according to their chemical structure (8, 9).

The intake of flavonols and flavones assessed around 1960, in a variety of countries, ranged from 3 mg/day in Finland to 70 mg/day in Japan (10). In 1976, the average daily intake of flavonoids in the United States was estimated as ~1 g, expressed as glycosides (quercitrin), of which 160–175 mg consisted of flavonols, flavanones, and flavones (11). These estimates were based on techniques now considered to be inappropriate and inaccurate (12).

In The Netherlands, in 1987–1988 the average intake was estimated as 23 mg/day, from an analysis of the population's commonly consumed vegetables and beverages, although restricted to selected flavonols and flavones (13). There are no

data about the flavonoid content of vegetable foods consumed in Brazil (14). This work reports both the composition and the content of flavonoids found in commercial vegetables and fruits commonly consumed by the Brazilian population. The effect of time of harvesting on flavonoid content was also studied, and an estimate of flavonoid daily intake by Brazilians was made for the first time.

### MATERIALS AND METHODS

**Materials.** Data sources on food frequency consumption were obtained from IBGE–Institute of Statistics Family Budget Surveys (15) carried out from October 1995 to September 1996 (16014 households) in 11 metropolitan areas of Brazil: Belém, Fortaleza, Recife, Salvador, Belo Horizonte, Rio de Janeiro, São Paulo, Curitiba, Porto Alegre, Brasília-DF, and Goiânia. Information about sources on food frequency consumption was also obtained in a Multicentric Study of Food Consumption carried out by INAN–National Institute of Food and Nutrition (16) in 1996, in five cities of Brazil. This information was complemented by the Annual Statistics Commercial Report of the São Paulo Central Market–CEAGESP (17) in 1999, which represents ~30% of the total Brazilian vegetable market.

All fresh vegetables and fruits (1–2 kg each) were purchased in the São Paulo Central Market (CEAGESP) at the time of their most frequent consumption, during two periods: the second semester of 2001 and the first semester of 2002. After purchase, the edible and nonedible parts of the samples were immediately cleaned, chopped into small pieces, frozen in liquid nitrogen, and stored at –80 °C until the time of analysis. At the time of analysis, samples were thoroughly homogenized by powdering in liquid nitrogen. Moisture was measured by drying at 105 °C and at 70 °C under vacuum, according to the AOAC *Methods of Analysis* (18).

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**Table 1.** Flavonoid Content (Milligrams per 100 g of FW) of Vegetables (Edible Portion) Sampled in the Second Semester of 2001, Expressed as Aglycons<sup>a</sup>

| sample                                       | % H <sub>2</sub> O | quercetin  | kaempferol      | luteolin  | apigenin  | cyanidin   | total |
|--|--------------------|------------|-----------------|-----------|-----------|------------|-------|
| <i>Lactuca sativa</i> L.                     |                    |            |                 |           |           |            |       |
| smooth lettuce                               | 94.7 ± 0.4         | 3.2 ± 0.3  | nd <sup>b</sup> | 1.0 ± 0.0 | nd        | nd         | 4.2   |
| rough lettuce                                | 94.7 ± 0.4         | 18.4 ± 0.3 | nd              | 0.2 ± 0.0 | nd        | nd         | 18.6  |
| red lettuce                                  | 94.2 ± 0.2         | 44.9 ± 2.5 | nd              | 3.1 ± 0.1 | nd        | 19.0 ± 0.7 | 67.0  |
| <i>Capsicum annuum</i> L. var. <i>annuum</i> |                    |            |                 |           |           |            |       |
| red pepper                                   | 92.2 ± 0.2         | 1.2 ± 0.2  | nd              | 0.6 ± 0.0 | nd        | nd         | 1.8   |
| yellow pepper                                | 92.8 ± 0.0         | 0.8 ± 0.0  | nd              | 0.9 ± 0.0 | nd        | nd         | 1.7   |
| green pepper                                 | 93.6 ± 0.3         | 4.1 ± 0.5  | nd              | 2.1 ± 0.2 | nd        | nd         | 6.2   |
| <i>Allium cepa</i> L. var. <i>cepa</i>       |                    |            |                 |           |           |            |       |
| white onion                                  | 91.5 ± 0.2         | 55.6 ± 2.0 | nd              | nd        | nd        | nd         | 55.6  |
| red onion                                    | 88.5 ± 0.6         | 38.3 ± 1.1 | nd              | nd        | nd        | 1.6 ± 0.1  | 39.9  |
| <i>Cichorium intybus</i> L.                  |                    |            |                 |           |           |            |       |
| chicory                                      | 91.8 ± 0.1         | 25.2 ± 3.4 | 11.1 ± 2.2      | nd        | 1.8 ± 0.1 | nd         | 38.1  |
| <i>Eruca sativa</i> Mill.                    |                    |            |                 |           |           |            |       |
| arugula                                      | 92.0 ± 0.2         | 13.9 ± 1.1 | 104.2 ± 3.4     | nd        | nd        | nd         | 118.1 |

<sup>a</sup> Values are means ± SD. <sup>b</sup> Not detected.

**Table 2.** Flavonoid Content (Milligrams per 100 g of FW) of Vegetables (Edible Portion) Sampled in the First Semester of 2002, Expressed as Aglycons<sup>a</sup>

| sample                                       | % H <sub>2</sub> O | quercetin  | kaempferol      | luteolin  | apigenin  | cyanidin   | total |
|--|--------------------|------------|-----------------|-----------|-----------|------------|-------|
| <i>Lactuca sativa</i> L.                     |                    |            |                 |           |           |            |       |
| smooth lettuce                               | 96.0 ± 0.2         | 2.2 ± 0.0  | nd <sup>b</sup> | 0.1 ± 0.0 | nd        | nd         | 2.3   |
| rough lettuce                                | 94.5 ± 0.3         | 20.6 ± 1.1 | nd              | 0.2 ± 0.0 | nd        | nd         | 20.8  |
| red lettuce                                  | 95.3 ± 0.0         | 37.5 ± 1.9 | nd              | 8.8 ± 0.9 | nd        | 20.8 ± 1.5 | 67.1  |
| <i>Capsicum annuum</i> L. var. <i>annuum</i> |                    |            |                 |           |           |            |       |
| red pepper                                   | 93.1 ± 0.2         | 0.3 ± 0.0  | nd              | 0.5 ± 0.0 | nd        | nd         | 0.8   |
| yellow pepper                                | 91.3 ± 0.2         | 2.0 ± 0.0  | nd              | 1.1 ± 0.0 | nd        | nd         | 3.1   |
| green pepper                                 | 93.4 ± 0.0         | 1.8 ± 0.0  | nd              | 1.2 ± 0.2 | nd        | nd         | 3.0   |
| <i>Allium cepa</i> L. var. <i>cepa</i>       |                    |            |                 |           |           |            |       |
| white onion                                  | 89.8 ± 0.0         | 48.2 ± 1.1 | nd              | nd        | nd        | nd         | 48.2  |
| red onion                                    | 87.8 ± 0.2         | 93.6 ± 3.5 | nd              | nd        | nd        | 6.1 ± 0.3  | 99.7  |
| <i>Cichorium intybus</i> L.                  |                    |            |                 |           |           |            |       |
| chicory                                      | 93.2 ± 0.2         | 3.7 ± 0.5  | 3.6 ± 0.4       | 7.8 ± 0.1 | 2.8 ± 0.3 | nd         | 17.9  |
| <i>Eruca sativa</i> Mill.                    |                    |            |                 |           |           |            |       |
| arugula                                      | 94.2 ± 0.1         | nd         | 40.7 ± 0.3      | nd        | nd        | nd         | 40.7  |

<sup>a</sup> Values are means ± SD. <sup>b</sup> Not detected.

**Chemicals.** All chemicals and solvents were of reagent or HPLC grade. The standards of luteolin, apigenin, kaempferol, quercetin, catechin, and epicatechin were obtained from Sigma Chemical Co. (St. Louis, MO); the standards of cyanidin, pelargonidin, delphinidin, malvidin, and peonidin were from Extrasynthese (Genay, France); naringenin, hesperetin, and chalcones chalconaringenin and phloridzin were from Plantech (Reading, U.K.); cyanidin 3-glycoside and naringin were from Apin Chemicals (Abingdon, U.K.); and rutin was from Merck (Darmstadt, Germany).

**Flavonoid extraction** was performed according to the method of Price et al. (19) with slight modifications, as follows. Samples were thoroughly homogenized by powdering in liquid nitrogen. Duplicate samples of the fresh powder (20 g) were extracted three times in methanol/water (100 mL the first time, 50 mL the next two times) or methanol/water/acetic acid (for anthocyanin samples) with a final solvent composition of 70:30 or 70:30:5, including the water of samples, at speed five for 1 min (Brinkmann homogenizer, Polytron—Kinematica GmbH, Kriens-Luzern, Sweden), in an ice bath. The homogenate was filtered under reduced pressure through filter paper (Whatman no. 06).

The extracts obtained were concentrated until methanol elimination on a rotatory evaporator (Rotavapor RE 120, Büchi, Flawil, Sweden) at ≤40 °C and made up to 25 mL with water for posterior application to solid-phase extraction (SPE) columns.

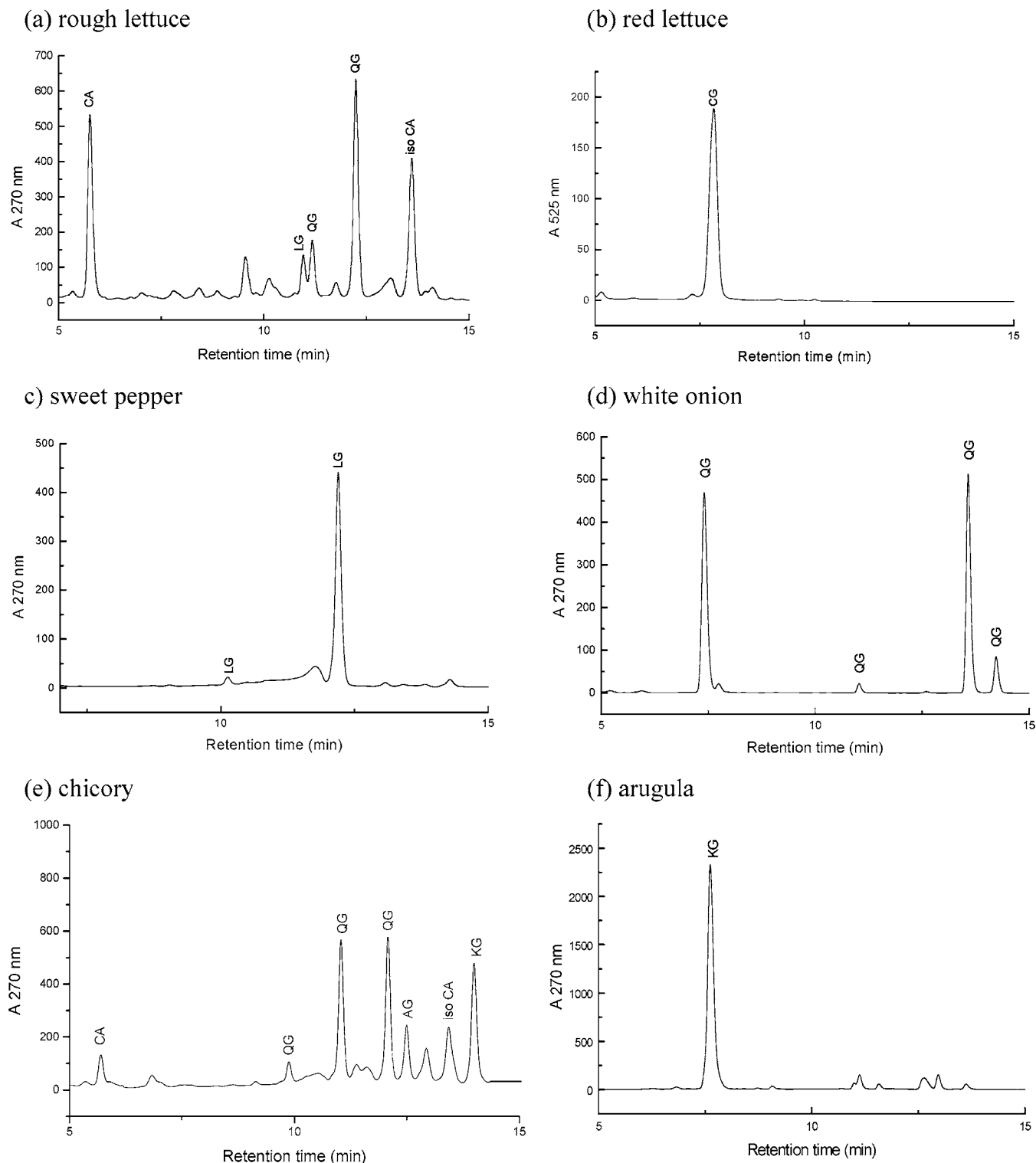
**Solid-Phase Extraction.** Aliquots (10 mL) of the extracts obtained above were passed through polyamide SC 6 (Macherey-Nagel GmbH and Co., Duren, Germany) columns (1 g/6 mL) previously conditioned with 20 mL of methanol and 60 mL of water. The columns were washed with water (20 mL) and eluted with 50 mL of methanol followed by 50 mL of 99.5:0.5 methanol/ammonia. Each eluate was evaporated to

dryness under reduced pressure at 40 °C, redissolved in methanol or methanol/acetic acid 95:5 (1 mL), and filtered through a 0.22 μm poly-(tetrafluoroethylene) (PTFE) filter (Millipore Ltd., Bedford, MA) prior to HPLC analysis.

**Analytical HPLC.** Identification and quantification of flavonoids was achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with an autosampler and a quaternary pump coupled to a diode array detector. The column used was a Prodigy 5 μ ODS 3 reversed-phase silica (250 mm × 4.6 mm i.d., Phenomenex Ltd.), and elution solvents were A (water/tetrahydrofuran/trifluoroacetic acid 98:2:0.1) and B (acetonitrile). The solvent gradient was the same as that used by Price et al. (19). Eluates were monitored at 270, 370, and 525 nm. Samples were injected in duplicate, and flavonoids were quantified using the respective external standards. Flavonoid standard solutions were prepared by dissolving in HPLC grade methanol and stored at −20 °C between analyses. Calibration was performed by injecting the standard three times at five different concentrations. Peak identification was performed by comparison of retention times and diode array spectral characteristics with the standards and the library spectra. Cochromatography was used when necessary. Quantification of quercetin glycosides was performed using quercetin as the standard, except for rutin, for which the rutin standard was used. Results were expressed as milligrams of aglycon per 100 g of fresh weight (FW), as mean ± standard deviation (SD).

## RESULTS AND DISCUSSION

**Tables 1** and **2** report the flavonoid content of fresh vegetables at two different periods of the year. Typical chro-



**Figure 1.** HPLC chromatograms of neutral glycosides of flavonoids extracted from (a) rough lettuce, (b) red lettuce, (c) sweet pepper, (d) white onion, (e) chicory, and (f) arugula. CA, chlorogenic acid; isoCA, isochlorogenic acid; CG, cyanidin glycosides; QG, quercetin glycosides; LG, luteolin glycosides; KG, kaempferol glycosides; AG, apigenin glycosides.

matograms are shown in **Figure 1**. As can be noted, the most widespread flavonoid was the flavonol quercetin, always present in glycosylated forms. Onion, lettuce, and arugula were the samples in which the largest concentrations of total flavonoids were found.

**Effect of Time of Harvesting.** In our study, it was observed that the total flavonoid content due to time of harvesting was quite variable for vegetables such as chicory (18 and 38 mg/100 g of FW), arugula (41 and 118 mg/100 g of FW), and

smooth lettuce (2 and 4 mg/100 g of FW), which presented much higher flavonoid levels during the second semester of 2001 compared with the first semester of 2002. Seasonal variability was, however, low for rough and red lettuces (**Tables 1** and **2**). Hertog et al. (7) observed that seasonal variability was large for leafy vegetables such as lettuce (cv. Capitata), endive, and leek, with flavonoid quantities 3–5 times higher during the summer than in other seasons due to a higher incidence of light. Similar to our results, they did not observe this variation in

**Table 3.** Flavonoid Content (Milligrams per 100 g of FW) of Tomatoes (Edible Portion) Sampled in the Second Semester of 2001, Expressed as Aglycons<sup>a</sup>

| sample  | % H <sub>2</sub> O | quercetin | kaempferol      | luteolin | chalconaringenin | total |
|---|--------------------|-----------|-----------------|----------|------------------|-------|
| <i>Lycopersicon esculentum</i> Mill.                  |                    |           |                 |          |                  |       |
| salad tomato var. <i>esculentum</i>                   | 94.1 ± 0.0         | 0.5 ± 0.0 | nd <sup>b</sup> | nd       | 1.7 ± 0.1        | 2.2   |
| Caqui tomato cv. Momotaro                             | 94.4 ± 0.0         | 1.3 ± 0.1 | nd              | nd       | 1.3 ± 0.0        | 2.6   |
| cherry tomato var. <i>cerasiforme</i> (Dunal) A. Gray | 91.6 ± 0.4         | 4.2 ± 0.6 | nd              | nd       | 4.0 ± 0.2        | 8.2   |

<sup>a</sup> Values are means ± SD. <sup>b</sup> Not detected.

**Table 4.** Flavonoid Content (Milligrams per 100 g of FW) of Oranges (Edible Portion) Sampled in the First Semester of 2002, Expressed as Aglycons<sup>a</sup>

| sample                             | % H <sub>2</sub> O | quercetin | sinensetin      | naringenin | hesperetin  | total |
|------------------------------------|--------------------|-----------|-----------------|------------|-------------|-------|
| <i>Citrus sinensis</i> (L.) Osbeck |                    |           |                 |            |             |       |
| Pera orange, pulp                  | 83.3 ± 1.2         | 0.9 ± 0.0 | nd <sup>b</sup> | 17.0 ± 0.8 | 16.9 ± 1.9  | 34.8  |
| Lima orange, pulp                  | 85.2 ± 1.3         | 0.8 ± 0.0 | nd              | 28.6 ± 0.2 | 14.9 ± 0.9  | 44.3  |
| Pera orange, skin                  | 71.9 ± 0.4         | 4.1 ± 0.0 | 19.6 ± 0.4      | 37.4 ± 1.5 | 109.4 ± 5.1 | 170.5 |
| Lima orange, skin                  | 73.9 ± 0.1         | 3.6 ± 0.1 | 17.0 ± 1.2      | 25.1 ± 1.1 | 79.7 ± 0.3  | 125.4 |

<sup>a</sup> Values are means ± SD. <sup>b</sup> Not detected.

flavonoid content for white onions. Red onions, however, presented a flavonoid content in the second semester more than twice that found in the first semester of the year (Tables 1 and 2). Peppers did not show a clear tendency to increase flavonoid content. According to Dupont et al. (20) variations within a variety could be due to differing agronomic conditions, tissue type (red to green), and leaves sampled (outer or inner).

**Flavonoids in Lettuce.** The individual types of lettuce exhibited very wide differences in total flavonoid content, which ranged from 2 to 4 mg/100 g of FW for the smooth, from 18 to 21 mg/100 g of FW for the rough, and ~67 mg/100 g of FW for the red lettuce. As can be seen from Tables 1 and 2, the higher flavonoid content of red lettuce cannot be accounted for only by the presence of anthocyanins (expressed as the corresponding aglycon, cyanidin), but must be due also to a much higher quercetin content (present as glycosides). Cyanidin contributed only 28–31% of the total flavonoid content of red lettuce, whereas quercetin contributed 56–67%. In the study of Dupont et al. (20) the cyanidin conjugates in two red-leafed varieties contributed 13 and 17% of the total flavonoid content, respectively. The higher flavonoid content of red lettuce in relation to green lettuce has already been reported by other authors (20, 21). In the study of Dupont et al. (20) quercetin was found in concentrations of 0–23 mg/100 g of FW for green lettuce and 5.6–15.4 mg/100 g of FW for the red one. Crozier et al. (21) reported much higher quercetin concentrations in red lettuce Lollo Rosso (45–91 mg/100 g of FW) when compared with green lettuce Lollo Bionda (9.4 mg/100 g of FW) and attributed this to the proximity of the flavonol and anthocyanin biosynthesis pathways.

A small amount of the flavone luteolin was also detected. The mean luteolin content of lettuce was of 2 mg/100 g of FW. These results are similar to those of Dupont et al. (20), 0.01–2.3 mg/100 g of FW. Hertog et al. (7) could not detect any luteolin in lettuce.

**Flavonoids in Sweet Pepper.** Sweet pepper, like lettuce, contained both quercetin and luteolin conjugates. As can be seen in Tables 1 and 2, quercetin conjugates, in a range of 0.3–4.1 mg/100 g of FW, and luteolin conjugates, in a range of 0.5–2.1 mg/100 g of FW, were detected. Lugasi and Hóvári (22) found 0.9 mg/100 g of FW of quercetin conjugates and 1.1 mg/100 g of FW of luteolin conjugates in different types of sweet pepper. Our present results are higher than those of Justesen et

al. (23), who detected 0.1, 0.2, and 0.5 mg of luteolin per 100 g of FW in red, yellow, and green sweet peppers, respectively. Hertog et al. (7) found luteolin only in red bell pepper, at a level of 1.1 mg/100 g of FW.

**Flavonoids in Onion.** Significant amounts of quercetin conjugates were detected in onion types. In white onions, quercetin conjugates were detected in concentrations of 48 and 56 mg of aglycon/100 g of FW, which was in the ranges of 19–63 mg/100 g of FW reported by Crozier et al. (21) and 28–49 mg/100 g of FW reported by Hertog et al. (7).

In red onions, 38 and 94 mg/100 g of FW of quercetin were detected, depending on the time of harvesting. Rhodes and Price (24) reported values of 92 mg of quercetin/100 g of FW in red onions and 71 and 80 mg/100 g of FW for pink and brown onions, respectively. They found that cyanidin conjugates in the red onion varieties contributed 9.2% of the total flavonoid content, almost 2 times higher than the values reported here (~5%). According to the compilation from the literature made by Clifford (25), in red onions the content of anthocyanin was up to 25 mg/100 g.

**Flavonoids in Dark Green Leaves.** Significant amounts of the flavonol kaempferol were detected in the samples of bitter leaves. As can be seen in Tables 1 and 2, arugula was the sample in which the largest concentrations of kaempferol were found (41 and 104 mg/100 g of FW), and quercetin derivatives were absent or in a much lower concentration (14 mg/100 g of FW), depending on time of harvesting. Chicory, on the contrary, presented similar amounts of quercetin (4–25 mg/100 g of FW) and kaempferol (4–11 mg/100 g of FW) glycosides but presented also apigenin and luteolin derivatives. According to Dupont et al. (20), although *Lactuca* and *Chicorium* species are both from the Compositae family, they have distinct profiles. Hertog et al. (7) reported very low levels, below 1 and 2 mg/100 g of FW, of quercetin and kaempferol derivatives, respectively, in chicory.

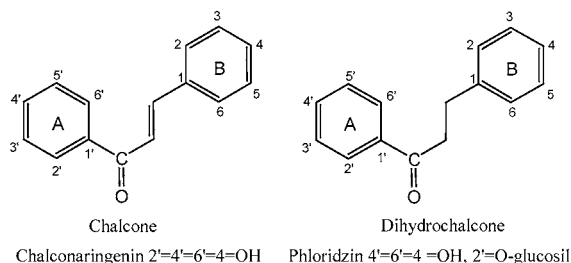
Tables 3–5 report the flavonoid content of fresh tomatoes, oranges, and apples. Similar to fresh vegetables, the most widespread flavonoid in the fruits analyzed was the flavonol quercetin, always present in glycosylated forms.

**Flavonoids in Tomato.** A large proportion of tomato phenolics occurs in the cuticle of the fruit (12), and as most tomato cultivars are normally consumed with the peel they were analyzed as a whole. Significant amounts of quercetin were

**Table 5.** Flavonoid Content (Milligrams per 100 g of FW) of Apples (Edible Portion) Sampled in the Second Semester of 2001, Expressed as Aglycons<sup>a</sup>

| sample                         | % H <sub>2</sub> O | quercetin  | epicatechin | catechin  | cyanidin        | phloridzin | total |
|--------------------------------|--------------------|------------|-------------|-----------|-----------------|------------|-------|
| <i>Malus domestica</i> Borkh   |                    |            |             |           |                 |            |       |
| Gala apple (red)               | 86.8 ± 0.1         | 10.1 ± 1.2 | 10.4 ± 0.4  | 5.1 ± 0.8 | nd <sup>b</sup> | 2.1 ± 0.0  | 27.7  |
| Fuji apple (red)               | 82.3 ± 0.6         | 0.4 ± 0.0  | 5.4 ± 0.1   | 1.3 ± 0.0 | nd              | 2.0 ± 0.0  | 9.1   |
| Golden Delicious apple (green) | 84.2 ± 0.2         | 2.3 ± 0.2  | 5.5 ± 0.3   | 1.6 ± 0.3 | nd              | 2.3 ± 0.0  | 11.7  |

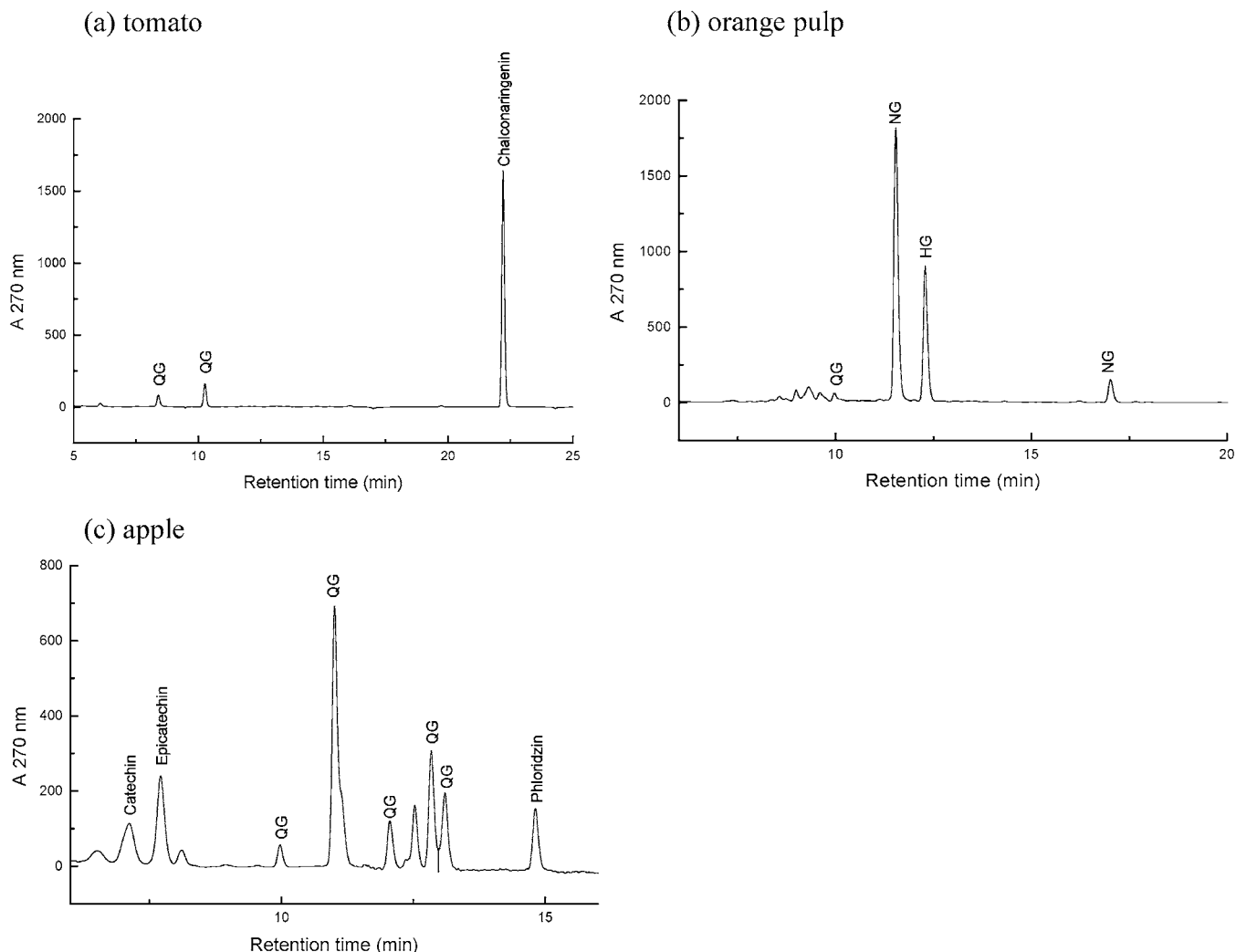
<sup>a</sup> Values are means ± SD. <sup>b</sup> Not detected.

**Figure 2.** Basic structures of chalcones.

detected. In ripe salad and caqui tomatoes quercetin was detected in levels of 0.5 and 1.3 mg/100 g of FW, respectively. However, ripe cherry tomatoes presented a much higher content of 4.2 mg of quercetin/100 g of FW (**Table 3**). Our results are similar

to those found by Crozier et al. (21), who observed that quercetin levels varied greatly between normal cultivars (0.2–1.2 mg/100 g of FW) and cherry tomatoes (0.2–20 mg/100 g of FW). The main classes identified in the cuticles of tomato cultivars were the quercetin derivatives and the chalcone chalconaringenin (**Figure 2**). A typical chromatogram is shown in **Figure 3**. According to **Table 3**, chalconaringenin levels were similar in salad and caqui tomatoes but in cherry tomato were ~1.5 times higher. According to Tomás-Barberán and Clifford (26), chalcones have restricted occurrence in foods. Chalconaringenin is present in tomato peel and may be present in tomato products. Acid hydrolysis converts the chalcone to the corresponding flavanone (naringenin), naturally present only in trace amounts.

According to Robards et al. (12), the composition of the flavonoid fraction is controlled by the spectral quality of incident

**Figure 3.** HPLC chromatograms of neutral glycosides of flavonoids extracted from (a) tomato, (b) orange pulp, and (c) apple. QG, quercetin glycosides; NG, naringenin glycosides; HG, hesperitin glycosides.



**Table 6.** Estimated Flavonoid Ingestion in Typical Brazilian Diets

| estimated ingestion of flavonoids            | food sources (%) <sup>a</sup>                      | population assessed   | ref |
|--|--|---|-----|
| 74.4 mg/day                                  | orange (70.2%)<br>lettuce (8.9%)<br>onion (5.8%)   | demographic census/SP   | 39  |
| 59.5 mg/day for women<br>77.1 mg/day for men | orange (70%)<br>lettuce (8.3%)<br>tomato (2.3%)    | 559 adults older than 20 years<br>living in São Paulo                   | 40  |
| 106.3 mg/day                                 | orange (47.1%)<br>arugula (30%)<br>lettuce (7.4%)  | 200 adults from 35 to 69 years, in a<br>case-control study in São Paulo | 41  |
| 74.2 mg/day                                  | orange (70.2%)<br>lettuce (11.7%)<br>tomato (2.6%) | 145 female students, aged 17–25 years,<br>from the city of Campinas, SP | 42  |

<sup>a</sup> Percentage of the total estimated ingestion.

radiation, with red light favoring the formation of chalconaringenin. Stewart et al. (27) observed that the flavonoid content of tomatoes varies due to different cultivars, size, and agronomic and climatic conditions. Muir et al. (28) developed transgenic tomatoes in which flavonol biosynthesis was up-regulated to generate ripe fruits with increased levels of flavonols, to increase their antioxidant capacity and potential health benefits. According to them, fruit flesh did accumulate significant amount of flavonoids. They also observed that the levels of rutin in tomato fruit peel increased during ripening and that chalconaringenin was absent in green peel but increased sharply during coloring of the fruit. Colliver et al. (29) demonstrated different routes to up-regulate flavonoid biosynthesis in tomatoes. In peel tissue, chalcone isomerase gene activity was shown to be critical, leading to a large increase in the level of quercetin glycoside accumulation. Le Gall et al. (30) detected a total flavonoid glycoside content of ripe transgenic tomatoes of ~10-fold higher than that of the controls, and kaempferol glycosides accounted for 60% of this. According to them, kaempferol glycosides comprised ~5% of the flavonoid glycoside of ripe control tomatoes.

**Flavonoids in Orange.** Flavanones are the flavonoids predominant in citrus (Figure 3). The flavanones naringenin and hesperetin were present in the pulp and peel of oranges. The amounts of flavanone present in the peel (105 and 147 mg/100 g of FW) were ~3 times higher than in the pulp (34 and 44 mg/100 g of FW) for Pera and Lima varieties, respectively. Larrauri et al. (31) detected the flavanones naringenin and hesperetin in the peel of orange, the last one being the major component. These compounds were also observed in commercial orange juices, which are obtained by pressing the whole fruit along with the peel (32–34). Justesen et al. (23) reported naringenin and hesperetin contents of orange pulp to average 11 and 31 mg/100 g of FW.

Quercetin derivatives were detected as minor components, in amounts of around 1 and 4 mg/100 g of pulp and peel, respectively (Table 4).

Citrus fruits are a special case because they contain a number of polymethoxylated flavones as minor flavonoids, such as nobiletin and sinensetin, in sweet orange peel (12). We detected sinensetin only in the peel of orange, in amounts of ~18 mg/100 g of FW. According to a review by Drewnowski and Gomez-Carneros (35), a small amount of the bitter flavone sinensetin, ~0.1 mg/L, could be found in fresh orange juice.

**Flavonoids in Apple.** In this study, we analyzed apples without removing their skin and found values for quercetin (0.4–10.1 mg/100 g of FW) presented in Table 5, similar to the 2.6–7.4 mg/100 g of FW reported by Price et al. (19). They

verified that peeling of the fruit reduced the flavonol content to very low levels and that the individual flavonol conjugates are not necessarily distributed in the same proportions between the flesh and peel of the fruit.

Flavanols (catechin and epicatechin) are present in high concentrations in apples (Figure 3). According to Table 5, catechin and epicatechin were found in different apple varieties in different proportion among them (1.3–5.1 mg/100 g of FW for catechin and 5.4–10.4 mg/100 g of FW for epicatechin). Arts et al. (36) reported much higher contents of epicatechin (7–10 mg/100 g of FW) compared to catechin (0.5 mg/100 g of FW) in seven apples varieties analyzed with the skin. They observed that removing the apple skin resulted, on average, in a 23% decrease of total catechin content.

Flavanols and dihydrochalcones (phloridzin) are present only in minor quantities but distinguish apple from other fruits (Figure 2). In red cultivars, anthocyanins derived from cyanidin are present, too, mainly cyanidin 3-galactoside (12, 37), but were not detected in the whole fruit as they are mainly located in the skin. In this study the level of phloridzin was constant between apple varieties (~2 mg/100 g of FW). According to Awad et al. (37), the level of phloridzin increased from the skin to the seeds, and it was the main flavonoid in the seeds, where it contributed 98% of the total flavonoid content. Nakamura et al. (38) examined the antioxidant activities of dihydrochalcones and observed that they have a significant radical scavenging activity and thus suppress lipid peroxidation.

In general, our values are somewhat higher than those reported earlier by Hertog et al. (7) and Justesen et al. (23). These discrepancies may be due to different cultivars, varieties, and methodologies used, mostly because these authors failed to detect the presence of luteolin in the hydrolyzed extracts of lettuce and chalconaringenin in the hydrolyzed extracts of tomato, for example.

In summary, we were able to determine the flavanols quercetin and kaempferol, the flavones luteolin, apigenin, and sinensetin, the chalcones naringenin and phloridzin, the flavanones naringenin and hesperetin, and catechins in vegetables and fruits commonly consumed by Brazilian people. Quercetin was by far the most important flavonol, followed by the anthocyanins derived from cyanidin. Flavones, chalcones, and catechins were found only in restricted vegetables.

**Estimated Ingestion in Typical Brazilian Diets.** To estimate the intake of flavonoids in the adult Brazilian population, we estimated food consumption based on the diet composition obtained from several dietary surveys available. The first, Barretto et al. (39), was drawn from a list of basic foodstuffs in which they used a demographic census to establish the “refer-

ence family” and the DIEESE & PROCON Food Basket, which represents a list of basic foodstuffs, was used to assess minimum food expenditure. According to these results (Table 6), the total intake of flavonoids for a family composed of four members was, on average, 74.4 mg per day per person. Major sources of flavonoids were orange (70.2%), lettuce (8.9%), and onion (5.8%).

Second, we used the dietary food consumption for an adult population sample of 559 adults, older than 20 years, in the study of Mattos and Martins (40). According to Table 6, the total intake of flavonoids for this population would be, on average, 59.5 mg per day for women and 77.1 mg per day for men. Major sources of flavonoids were found in orange (70%), lettuce (8.3%), and tomato (2.3%).

Tomita and Cardoso (41) examined the food list and portion values from a Food Frequency Questionnaire among participants in a case-control study in São Paulo, Brazil. Two hundred adults were randomly selected with their ages ranging from 35 to 69 years old. We verified that the total intake of flavonoids for this population (Table 6) would be higher than for the others mentioned and ~106.3 mg per day. The major sources of flavonoids were orange (47.1%), arugula (30%), and lettuce (7.4%).

Finally, to estimate the intake of flavonoids in young women, we assessed food ingestion on the basis of a study of the dietary habits of 145 female students, aged 17–25 years, from the town of Campinas, São Paulo state (42). In this study their food habits, preferences, and consumption were assessed using a dietary history method. According to these results, the total intake of flavonoids for this population would be, on average, 74.2 mg per day (Table 6). Major sources of flavonoids were orange (70.2%), lettuce (11.7%), and tomato (2.6%).

From these results we can observe that even if ingestion varies according to the survey considered or with the population included, oranges represent the major flavonoid source, corresponding in three of the four studies to >70% of the total flavonoid ingestion, followed by lettuce, corresponding to 8–12%. Tomato and onion have lower significance; on the other hand, arugula, when present in the diet, is an important contribution to flavonoid ingestion. According to these considerations, we can conclude that the sources of flavonoids in the Brazilian diet are not very much diversified.

Recently, it was estimated that the intake of flavones, flavonols, and flavanones in Denmark was 28 mg per day (22). In Finland, the total average intake of 23 different flavonoid aglycons, estimated on the basis of food composition data, was 55.2 mg per day, with fruits contributing 36.5 mg per day followed by tea, wine, and other nonalcoholic beverages (22). Thus, our data indicated that Brazilian flavonoid intake could be significant and somewhat higher than in European countries even if the tea and wine consumption is much lower.

**Conclusion.** In this study we analyzed the flavonoid contents of a selection of vegetables frequently consumed in Brazil. Major sources of flavonoids were orange (70%), lettuce (9%), and tomato (2.5%). The most widespread compounds were the glycosides of quercetin, in a concentration that can vary according to the time of harvesting. These data provided a first basis for the assessment of the average intake of flavonoids by the Brazilian population aged 17–88, which averaged 79 mg per day for women and 86 mg per day for men.

## LITERATURE CITED

- (1) Caragay, A. B. Cancer: preventive foods and ingredients. *Food Technol.* **1992**, *46*, 65–68.

- (2) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer prevention: a review. *J. Am. Diet. Assoc.* **1996**, *96*, 1027–1039.
- (3) Hollman, P. C. H.; Katan, M. B. Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed. Pharmacother.* **1997**, *51*, 305–310.
- (4) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: Intake, health effects and bioavailability. *Food Chem. Toxicol.* **1999**, *37*, 937–942.
- (5) Harborne, J. B.; Williams, C. A. Advances in flavonoid research since 1992. *Phytochemistry* **2000**, *55*, 481–504.
- (6) Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: New York, 1982; 113 pp.
- (7) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in The Netherlands. *J. Agric. Food Chem.* **1992**, *40*, 2379–2383.
- (8) Cook, N. C.; Samman, S. Flavonoids—Chemistry, metabolism, cardioprotective effects, and dietary sources. *J. Nutr. Biochem.* **1996**, *07*, 66–76.
- (9) Peterson, J.; Dwyer, J. Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *J. Am. Diet. Assoc.* **1998**, *98*, 677–682.
- (10) Hollman, P. C. H.; Katan, M. B. Dietary flavonoids: intake, health effects and bioavailability. *Food Chem. Toxicol.* **1999**, *37*, 937–942.
- (11) Kuhnau, J. The flavonoids: a class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet.* **1976**, *24*, 117–191.
- (12) Robards, K.; Prenzler, P. D.; Tucker, G.; Swatsitang, P.; Glover, W. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* **1999**, *66*, 401–436.
- (13) Hertog, M. G. L.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **1993**, *20*, 21–29.
- (14) Lajolo, F. M. Functional foods: Latin American perspectives. *Brit. J. Nutr.* **2002**, *88*, S145–S150.
- (15) Instituto Brasileiro de Geografia e Estatística (IBGE). Ministério do Planejamento e Orçamento. *Pesquisa de Orçamentos Familiares 1995–1996: consumo alimentar domiciliar per capita: regiões Metropolitanas*; Município de Goiânia, Brazil, 1998; Vol. 2, 137 pp.
- (16) Instituto Nacional de Alimentos e Nutrição (INAN), Ministério da Saúde. *Estudo multicêntrico sobre consumo alimentar*; Documento Final; Campinas, 1996; n.p.
- (17) Companhia de Entrepósitos e Armazéns Gerais do Estado de São Paulo (CEAGESP). Ministério da Agricultura e do Abastecimento. *Boletim Anual – Dados Estatísticos relativos aos Produtos Hortigranjeiros e Pescado afluídos ao ETSP*; 1999; n.p.
- (18) AOAC. *Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Washington, DC, 1980.
- (19) Price, K. R.; Prosser, T.; Richein, A. M. F.; Rhodes, M. J. C. A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing. *Food Chem.* **1999**, *66*, 489–494.
- (20) Dupont, M. S.; Mondim, Z.; Williamson, G.; Price, K. R. Effect of Variety, Processing, and Storage on the Flavonoid Glycoside Content and Composition of Lettuce and Endive. *J. Agric. Food Chem.* **2000**, *48*, 3957–3964.
- (21) Crozier, A.; Lean, M. E. J.; McDonald, M. S.; Black, C. Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery. *J. Agric. Food Chem.* **1997**, *45*, 590–595.
- (22) Lugasi, A.; Hóvári, J. Flavonoid aglycons in foods of plant origin I Vegetables. *Acta Aliment.* **2000**, *29*, 345–352.
- (23) Justesen, U.; Knuthsen, P.; Leth, T. Quantitative analysis of flavonols, flavones, and flavanones in fruits, vegetables and beverages by high-performance liquid chromatography with photo-diode array and mass spectrometric detection. *J. Chromatogr. A* **1998**, *799*, 101–110.

- (24) Rhodes, M. J. C.; Price, K. R. Analytical problems in the study of flavonoid compound in onions. *Food Chem.* **1996**, *57*, 113–117.
- (25) Clifford, M. N. Review. Anthocyanins—nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1063–1072.
- (26) Tomás-Barberán, F. A.; Clifford, M. N. Review. Flavanones, chalcones, and dihydrochalcones—nature, occurrence and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1073–1080.
- (27) Stewart, A. J.; Bozonnet, S.; Mullen, W.; Jenkins, G. I.; Lean, M. E. J.; Crozier, A. Occurrence of flavonols in tomatoes and tomato-based products. *J. Agric. Food Chem.* **2000**, *48*, 2663–2669.
- (28) Muir, S. R.; Collins, G. J.; Robinson, S.; Hughes, S.; Bovy, A.; Ric de Vos, C. H.; Van Tunen, A. J.; Verhoeven, M. E. Overexpression of Petunia chalcone isomerase in tomato results in fruit containing increased levels of flavonols. *Nat. Biotechnol.* **2001**, *19*, 470–474.
- (29) Colliver, S.; Bovy, A.; Collins, G.; Muir, S.; Robinson, S.; Ric de Vos, C. H.; Verhoeven, M. E. Improving the nutritional content of tomatoes through reprogramming their flavonoid biosynthetic pathway. *Phytochem. Rev.* **2002**, *1*, 113–123.
- (30) Le Gall, G.; Dupont, M. S.; Mellon, F. A.; Davis, A. L.; Collins, G. J.; Verhoeven, M. E.; Colquhoun, I. J. Characterization and content of flavonoid glycosides in genetically modified tomato (*Lycopersicon esculentum*) fruits. *J. Agric. Food Chem.* **2003**, *51*, 2438–2446.
- (31) Larrauri, J. A.; Rupérez, P.; Bravo, L.; Saura-Calixto, F. High dietary fibre powders from orange and lime peels: associated polyphenols and antioxidant capacity. *Food Res. Int.* **1996**, *29*, 757–762.
- (32) Bronner, W. E.; Beecher, G. R. Extraction and measurement of prominent flavonoids in orange and grapefruit juice concentrates. *J. Chromatogr. A* **1995**, *705*, 247–256.
- (33) Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. Flavanone glycosides in Brazilian orange juice. *Food Chem.* **1998**, *61*, 275–280.
- (34) Leuzzi, U.; Caristi, C.; Panzera, V.; Licandro, G. Flavonoids in pigmented orange juice and second-pressure extracts. *J. Agric. Food Chem.* **2000**, *48*, 5501–5506.
- (35) Drewnowski, A.; Gomez-Careros, C. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.* **2000**, *72*, 1424–1435.
- (36) Arts, I. C. W.; Putte, B. V.; Hollman, P. C. H. Catechin contents of foods commonly consumed in The Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J. Agric. Food Chem.* **2000**, *48*, 1746–1751.
- (37) Awad, M. A.; Jager, A.; Westing, L. M. Flavonoid and chlorogenic acid levels in apple fruit: characterization of variation. *Sci. Hortic.* **2000**, *83*, 249–263.
- (38) Nakamura, Y.; Watanabe, S.; Miyake, N.; Kohno, H.; Osawa, T. Dihydrochalcones: evaluation as novel radical scavenging antioxidants. *J. Agric. Food Chem.* **2003**, *51*, 3309–3312.
- (39) Barretto, S. A. J.; Cyrillo, D. C.; Cozzolino, S. M. F. Análise nutricional e complementação alimentar de cesta básica derivada do consumo. *Rev. Saude Publica* **1998**, *32*, 29–35.
- (40) Mattos, L. L.; Martins, I. S. Consumo de fibras alimentares em população adulta. *Rev. Saude Publica* **2000**, *34*, 50–55.
- (41) Tomita, L. Y.; Cardoso, M. A. Avaliação da lista de alimentos e porções alimentares de Questionário Quantitativo de Frequência Alimentar em população adulta. *Cad. Saude Publica* **2002**, *18*, 1747–1756.
- (42) Sampaio, K. L.; Silva, M. A. A. P. Consumo, hábitos e preferências de jovens brasileiras universitárias. In *Congresso Brasileiro de Ciência e Tecnologia Alimentos*; Anais: Porto Alegre, Brazil, 2002; Vol. 18, pp 3665–3668.

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