

# Quantitative Analysis of the Flavonoid Content of Commercial Tomatoes, Onions, Lettuce, and Celery

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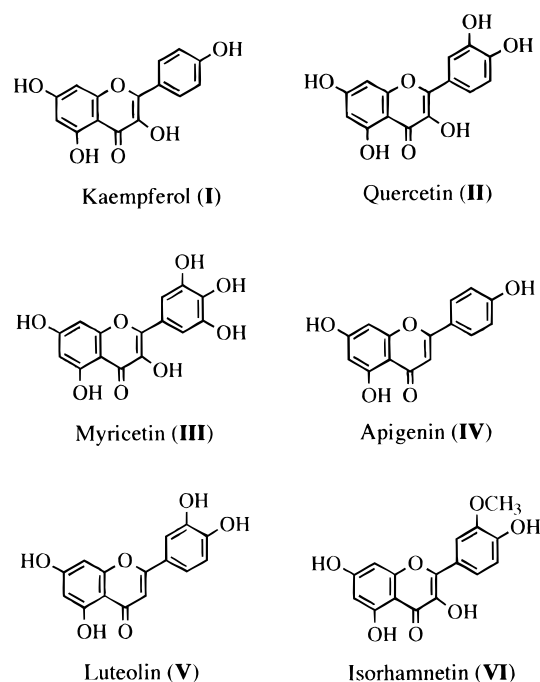
Quantitative estimates of conjugated flavonoid content were obtained by using HPLC to analyze the level of free flavonoids present in acid-hydrolyzed extracts from commercial fruits and vegetables. Cherry tomatoes contained 17–203  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  fresh weight compared to 2.2–11  $\mu\text{g}$   $\text{g}^{-1}$  detected in normal-sized Scottish, Spanish, and Dutch beef tomatoes. The quercetin levels in onions ranged from 185 to 634  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  fresh weight. "Round" lettuce contained 11  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  fresh weight compared to 911  $\mu\text{g}$   $\text{g}^{-1}$  in the outer leaves and 450  $\text{mg}$   $\text{g}^{-1}$  in the inner leaves of "Lollo Rosso" lettuce. The conjugated flavonoid content of celery was very variable, ranging from undetectable to 40  $\mu\text{g}$  of luteolin and 191  $\mu\text{g}$  of apigenin  $\text{g}^{-1}$  fresh weight. Cooking lowered the quercetin content of both tomatoes and onions with greater reductions being detected following microwaving and boiling than after frying.

**Keywords:** HPLC; quantitative analysis; flavonoids; tomatoes; onions; lettuce; celery; diet

## INTRODUCTION

Flavonoids are a large family of over 4000 secondary plant metabolites, comprising anthocyanins, flavonols, flavones, catechins, and flavonones (Harborne, 1994). Many flavonoids are present in plant tissues in relatively high concentrations as sugar conjugates. Most flavonoids, however, have very restricted distributions within the plant kingdom, many occurring in only one genus or even species (Markham, 1989). Among the more ubiquitous flavonoids over 50 different glycosides have been identified (Herrmann, 1976, 1988).

Flavonols and flavones are flavonoids of particular importance as they are have been found to possess antioxidant and free radical scavenging activity in foods (Shahidi and Wanasundara, 1992), and epidemiological studies have indicated that their consumption is associated with a reduced risk of cancer (Wattenburg, 1985, 1990; Verma *et al.*, 1988; Wei *et al.*, 1990) and cardiovascular disease (Gregory *et al.*, 1990; Hertog *et al.*, 1993a, 1994). Vegetables, fruits, and beverages are the main dietary sources of the flavonols, primarily as kaempferol (I), quercetin (II) and myricetin (III), and the corresponding flavones, apigenin (IV) and luteolin (V) (Figure 1) (Hertog *et al.*, 1992a, 1993b). The daily flavone and flavonol intake in the U.S.A., expressed as aglycones, was estimated to be >100 mg by Kühnau (1976). However, a more recent study with elderly Dutch patients has suggested a figure of ca. 25 mg per day (Hertog *et al.*, 1993a). One likely reason for this discrepancy is that the analytical methodology upon which the quantifications in the 1976 report were based was not sufficiently rigorous and yielded inaccurate over-estimates of flavonoid levels. In their studies, Hertog and co-workers (1992a,b, 1993b) made use of



**Figure 1.** Structures of kaempferol, quercetin, myricetin, apigenin, luteolin, and isorhamnetin.

carefully evaluated analytical methodology that involved extraction of plant tissues, acid hydrolysis of extracts to cleave flavonoid glycosides followed by the use of reverse phase high-performance liquid chromatography (HPLC) to identify and quantify the released aglycones.

The HPLC-based procedures for the quantitative analysis of endogenous flavonoids of Hertog *et al.* (1992b) have been further refined, with either kaempferol (I) or isorhamnetin (VI) (Figure 1) being used as internal standards to monitor losses during sample preparation (Crozier *et al.*, 1996). This paper reports on the use of these procedures to investigate seasonal and varietal differences in the flavonoid content of

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tomatoes, onion, and celery. In addition, flavonoid levels in commercial and home-grown varieties of lettuce were determined and the effects of cooking on the flavonoid content of tomatoes and onions were investigated. The data obtained from plant material acquired in the U.K. supplements the work of Hertog *et al.* (1992a) on flavonol and flavone content of fruits and vegetables commonly consumed in The Netherlands.

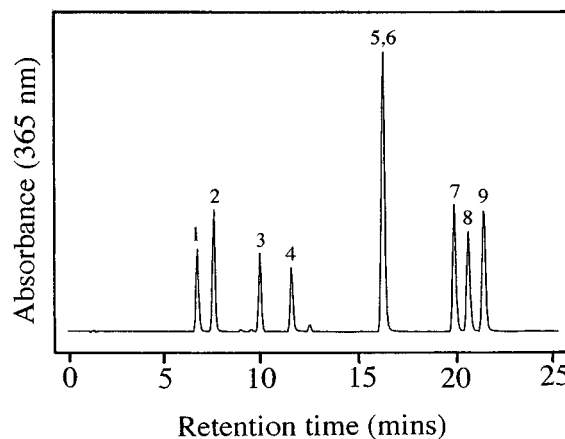
## MATERIALS AND METHODS

**Fruits and Vegetables.** Individual lettuce (*Lactuca sativa*) (country of origin, U.K.) and ca. 250 g quantities of tomatoes (*Lycopersicon esculentum* Mill.) (Spain, The Netherlands, Scotland, and England), red and white onions (*Allium cepa* L.) (U.K.), and celery (*Apium graveolens* L.) (U.K.) were selected at random from the fresh fruit and vegetable section of a local supermarket (Safeway, Byres Road, Glasgow G12, U.K.) between August 1994 and July 1995. Home-grown lettuce was obtained from a local garden in Milngavie, Glasgow. On the day of purchase/harvest, and after removing the dry outer scales from the onions and washing the tomatoes and other vegetables, the plant material was finely sliced and frozen in liquid nitrogen prior to lyophilization in an Edwards (Welynn Garden City, U.K.) Modulyo freeze-drier. When the tissue was freeze-dried, it was powdered with a pestle and mortar and stored at  $-20^{\circ}\text{C}$ . Fresh and dry weight measurements were obtained for all plant material.

**Cooking.** Samples of sliced Scottish grown tomatoes and onions, each ca. 10 g fresh weight, were (i) fried in 10 ml sunflower oil until cooked thoroughly, which took 2.5–3.0 min for tomatoes and 5 min for onions, (ii) boiled in 250 mL of simmering water for 15 min, and (iii) microwaved in a closed glass container with 10 mL of water in an 800 W oven until cooked which took 1.3 min for tomatoes and 2.5 min for onions. After drying with paper towels to remove water/sunflower oil, the cooked and uncooked materials were frozen in liquid nitrogen and freeze-dried as described above.

**Extraction and Hydrolysis Conditions.** Optimization of acidic conditions for the hydrolysis of flavonoid conjugates in a range of fruits, vegetables and beverages has been described by Hertog *et al.* (1992b) following an earlier detailed study by Harborne (1965) on the release of free flavonoids by acid and enzymic hydrolyses. In the present investigation, preliminary screenings were carried out to ascertain the most effective acid hydrolysis conditions for the tissues under study. The data obtained were in agreement with the findings of Hertog *et al.* (1992b). Thus, aliquots of 0.25 g of lyophilized tomato, onion and lettuce were extracted with 20 mL, 60% aqueous methanol containing 125  $\mu\text{g}$  of kaempferol as an internal standard and 20 mM sodium diethylthiocarbamate as an antioxidant. 5 mL of 6 M HCl was added to each extract to give a 25 mL solution of 1.2 M HCl in 50% aqueous methanol. Extracts were refluxed at  $90^{\circ}\text{C}$  for 2 h. Extract aliquots of 100  $\mu\text{L}$ , taken both before and after hydrolysis, were made up to 250  $\mu\text{L}$  with distilled water, adjusted to pH 2.5 with trifluoroacetic acid, and filtered through a 0.22  $\mu\text{m}$  filter prior to analysis of 50  $\mu\text{L}$  volumes (1/1250 aliquot of total sample) by reversed phase HPLC. Identical procedures were used with celery except that 125  $\mu\text{g}$  of isorhamnetin was used as an internal standard and samples were refluxed in 2 M HCl for 4 h at  $90^{\circ}\text{C}$ . In some instances, replicate lyophilized tissue samples were acid hydrolyzed and analyzed individually. Preliminary HPLC analyses were carried out with all tissues to establish that the kaempferol/isorhamnetin used as an internal standard was not present in detectable quantities in extracts either before or after acid hydrolysis.

**High-Performance Liquid Chromatography.** Samples were analyzed using a Shimadzu (Kyoto, Japan) LC-10A series automated liquid chromatograph comprising an SCL-10A system controller, two LC-10A pumps, a SIL-10A auto injector with sample cooler, a CTO-10A column oven and SPD-10A UV-vis detector linked to a Reeve Analytical (Glasgow, U.K.) 2700 data handling system. Reversed phase separations were carried out at  $40^{\circ}\text{C}$  using a  $150 \times 3.9$  mm i.d., 5  $\mu\text{m}$  C<sub>18</sub>



**Figure 2.** Gradient reversed phase HPLC analysis of free and conjugated flavonoids. Column:  $150 \times 3.9$  mm i.d., 5  $\mu\text{m}$  C<sub>18</sub> Symmetry. Mobile phase, a 20 min gradient of 15–35% acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid; flow rate, 1 mL min<sup>-1</sup>; detector, absorbance monitor operating at 365 nm. Samples: 100 ng of (1) rutin, (2) quercetin-3-glucoside, (3) quercitrin, (4) myricetin, (5) luteolin, (6) quercetin, (7) apigenin, (8) kaempferol, and (9) isorhamnetin.

Symmetry column fitted with a  $20 \times 3.9$  mm 5  $\mu\text{m}$  C<sub>18</sub> Symmetry guard column in an integrated sentry holder (Waters, Milford, MA). The mobile phase was a 20 min, 15–35% gradient of acetonitrile in water adjusted to pH 2.5 with trifluoroacetic acid, eluted at flow rate of 1 mL min<sup>-1</sup>. Column eluent was monitored at 365 nm (Crozier *et al.*, 1996). In selected instances, “stop-flow” scans were obtained with the UV-vis detector so that the 300–450 nm absorbance spectra of endogenous flavonoid peaks as well as the kaempferol and isorhamnetin internal standards could be compared with those of reference compounds. The acetonitrile-based gradient reversed phase HPLC system readily resolves all the flavonoid standards listed below except for luteolin and quercetin, which have very similar retention properties (Figure 2). All samples found to contain a quercetin/luteolin peak were therefore reanalyzed on the C<sub>18</sub> Symmetry column using a solvent of 50% methanol in water adjusted to pH 2.5 with trifluoroacetic acid. This is a modification of isocratic procedures originally described by Hertog *et al.* (1992b), which at a flow rate of 1 mL min<sup>-1</sup> provides rapid base line separation of quercetin ( $t_R$  4.9 min) and luteolin ( $t_R$  5.8 min) (Crozier *et al.*, 1996).

**Reference Compounds.** Apigenin, kaempferol, myricetin, quercetin, quercitrin (quercetin-3-L-rhamnoside), and rutin (quercetin-3 $\beta$ -D-rhamnoside) were purchased from Sigma Chemicals (Poole, Dorset, U.K.). Isorhamnetin, luteolin, and quercetin-3-glucoside were obtained from Apin Chemicals (Abingdon, Oxford, U.K.).

## RESULTS

**Analysis of Endogenous Flavonoids in Tomatoes.** None of the tomato extracts examined contained free flavonoids, but after acid hydrolysis, quercetin was detected. Quantitative estimates of the conjugated quercetin content of Scottish, Spanish, Dutch beef, Spanish cherry and English cherry tomatoes, purchased between August 1994 and June 1995 are presented in Table 1. There are marked differences in the quercetin content of different types of tomatoes. Lowest levels were found in Scottish, Spanish, and Dutch tomatoes, all of which are held in cold storage for varying periods after harvest and sold loose in large quantities by the major U.K. supermarkets. Dutch tomatoes (var. Trust) contained 2.2–6.8  $\mu\text{g g}^{-1}$ , while the quercetin content of the equivalent Spanish tomatoes (vars. Assun and Daniella) purchased between January and April 1995 ranged from 2.0 to 8.7  $\mu\text{g g}^{-1}$  fresh weight. Samples of

**Table 1. Conjugated Quercetin Content of Commercial Spanish, Scottish, Dutch Beef, Spanish Cherry, and English Cherry Tomatoes (*Lycopersicon esculentum* Mill.) Purchased at Different Dates between August 1994 and August 1995**

type	date of purchase	quercetin content ( $\mu\text{g g}^{-1}$ fresh weight)	
Spanish tomatoes var. Assun var. Assun var. Daniella var. Daniella	12 Jan 1995	4.4	
	23 Jan 1995	3.5	
	4 Feb 1995	2.0	
	3 Apr 1995	8.7	
	9 Jun 1995	$7.3 \pm 0.9^b$	
Scottish tomatoes var. Spectra	14 Jul 1995	4.8	
	28 Jul 1995	4.6	
	10 Aug 1995	11.2	
	3 Aug 1994 <sup>a</sup>	6.8	
Dutch beef tomatoes var. Trust	9 Jun 1995	$2.2 \pm 0.3^b$	
	14 Jul 1995	3.2	
	28 Jul 1995	3.5	
	10 Aug 1995	5.9	
	3 Aug 1994 <sup>a</sup>	38	
Spanish cherry tomatoes var. Paloma	12 Jan 1995	203	
	4 Feb 1995	179	
	3 Apr 1995	50	
	6 May 1995	55	
	9 Jun 1995	$55 \pm 2.9^b$	
	14 Jul 1995	28	
	28 Jul 1995	39	
	10 Aug 1995	23	
	English cherry tomatoes var. Favorita	4 Feb 1995	$77 \pm 1.8^b$
		9 Jun 1995	$41 \pm 0.5^b$
14 Jul 1995		17	
28 Jul 1995		32	
10 Aug 1995		40	

<sup>a</sup> No internal standard used with 3 Aug 1994 samples. <sup>b</sup> Standard error ( $n = 4$ ). Other estimates based on single samples. Overall mean values for the quercetin content of Spanish, Scottish, Dutch beef, Spanish cherry, and English cherry tomatoes are 4.6, 7.0, 4.3, 74, and  $41 \mu\text{g g}^{-1}$  fresh weight, respectively.

Scottish tomatoes (var. Spectra) purchased between June and August 1995 contained 4.6–11.2  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$ . In contrast to these low figures for large tomatoes, samples of Spanish cherry tomatoes (var. Paloma) purchased in January and February 1995 contained 203 and 179  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$ , respectively, while samples purchased in August 1994 and between April and August 1995 contained 23–55  $\mu\text{g g}^{-1}$ . English cherry tomatoes (var. Favorita) acquired between February and August 1995 also contained high levels of quercetin, although they were somewhat lower than Spanish cherry tomatoes purchased at the same time points.

The quercetin content of tomatoes obtained from a supermarket, a local grocery store, and a street market in the Netherlands, between April 1991 and April 1992, and analyzed by Hertog *et al.* (1992a) as part of the Zutphen Elderly Study (Hertog *et al.*, 1993a), ranged from 4.6 to 11  $\mu\text{g g}^{-1}$  fresh weight. This is similar to the levels detected in the present study in Spanish, Scottish, and Dutch tomatoes, but much lower than the quercetin content of both Spanish and English cherry tomatoes.

**Analysis of Endogenous Flavonoids in Onions.** Onions, like tomatoes, contained conjugated quercetin. Data obtained on the quercetin content of white onions and one sample of red onions, from which the dry outer skin had been removed, are presented in Table 2. Contrary to the results of Herrmann (1988) the red-skinned onion did not contain higher levels of quercetin than the white-skinned varieties. Although there is evidence of some seasonal variations in white onions, which were purchased between August 1994 and April 1995, the concentrations of quercetin were consistently high, ranging from 185 to 634  $\mu\text{g g}^{-1}$  fresh weight. These values are very similar to the 284–486  $\mu\text{g}$  of

**Table 2. Quercetin Content of Commercial Red and White Onions (*Allium cepa* L.) Purchased at Different Dates between August 1994 and April 1995**

type	purchase date	quercetin content ( $\mu\text{g g}^{-1}$ fresh weight)
red onion	3 Aug 1994 <sup>a</sup>	201
white onion	3 Aug 1994 <sup>a</sup>	332
	12 Jan 1995	185
	4 Feb 1995	634
	3 April 1995	331
	6 May 1995	$227 \pm 3.4^b$

<sup>a</sup> No internal standard used with 3 Aug 1994 samples. <sup>b</sup> Standard error ( $n = 3$ ). Other estimates based on single samples.

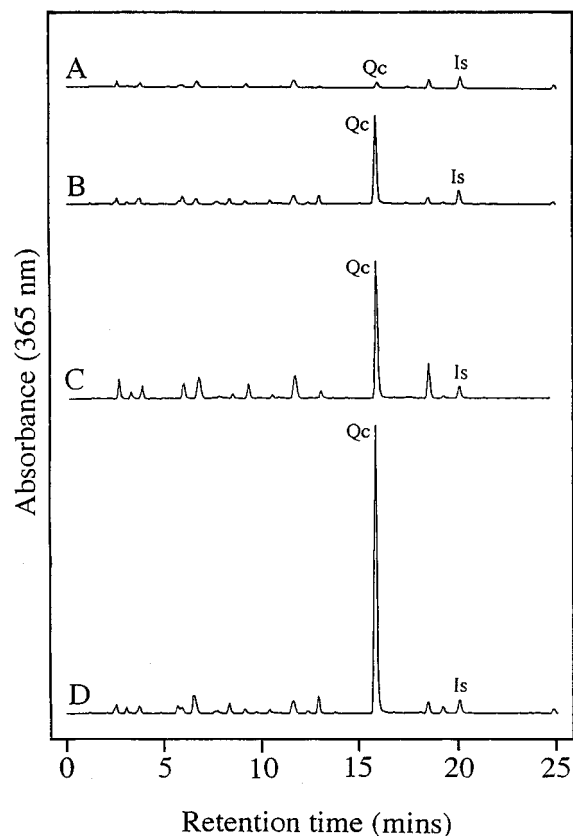
quercetin  $\text{g}^{-1}$  found in onions purchased commercially in The Netherlands (Hertog *et al.*, 1992a), but much higher than the values reported for U.S.-grown onions by Bilyk *et al.* (1984). In contrast to the findings of Bilyk *et al.* (1984), kaempferol was not detected in any of the onion extracts that were analyzed despite the limit of detection for kaempferol and other flavonoids being *ca.* 1  $\mu\text{g g}^{-1}$  fresh weight.

**Endogenous Flavonoids in Lettuce.** Like tomato and onion, lettuce contained conjugated quercetin and no free flavonoids were detected in extracts prior to acid hydrolysis. Information on the conjugated quercetin content of a number of commercial and home-grown lettuce is summarised in Table 3 and illustrated in Figure 3. The individual types of lettuce exhibited very wide differences in quercetin content. The "Round" lettuce, which is small, cheap, and sold in large quantities in U.K. supermarkets, contained only 11  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  fresh weight (Figure 3A). This contrasts with the 94  $\mu\text{g g}^{-1}$  in "Lollo Bionda" (Figure 3B) and the 450 and 911  $\mu\text{g g}^{-1}$  in the inner and outer leaves, respectively, of its red-leaved, anthocyanin-rich equivalent, "Lollo Rosso" (Figure 3C,D). The red-leaved, home-

**Table 3. Conjugated Quercetin Content of Different Varieties of Commercial and Home-Grown Lettuce (*Lactuca sativa* L.)<sup>a</sup>**

type/variety	source		quercetin content ( $\mu\text{g} \pm \text{SE}^b \text{g}^{-1}$ fresh weight)
"Round" lettuce var. Cortina	Safeway	whole lettuce	11 $\pm$ 0.5
"Green Salad Bowl"	home-grown	whole lettuce	147 $\pm$ 5.2
"Marvel of Four Seasons" (R)	home-grown	outer leaves	228 $\pm$ 29
		inner leaves	22 $\pm$ 2.3
"Lollo Rosso" var. Malibu (R)	Safeway	outer leaves	911 $\pm$ 27
		inner leaves	450 $\pm$ 17
"Lollo Bionda" var. Cerieo	Safeway	whole lettuce	94 $\pm$ 4.6

<sup>a</sup> Purchased/harvested in Glasgow on 28 July 1995. "(R)" indicates a variety with red leaves. <sup>b</sup>  $n = 3$ .



**Figure 3.** Gradient reversed phase HPLC analysis of flavonoids in lettuce. Column, mobile phase, flow rate, and detector as in Figure 2. Samples: 1/1250 aliquot of acid-hydrolyzed extracts from (A) "Round" lettuce, (B) "Lollo Bionda" lettuce, and (C) inner leaves and (D) outer leaves of "Lollo Rosso" lettuce. Qc, quercetin; Is, kaempferol internal standard.

grown "Marvel of Four Seasons" contained 228  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  in the outer leaves but there was a ca. 10-fold decline in the quercetin content of the inner leaves. The combined inner and outer leaves of the other home-grown cultivar "Green Salad Bowl" were relatively rich in quercetin, containing 147  $\mu\text{g}$   $\text{g}^{-1}$  fresh weight.

The quercetin content of the various lettuce cultivars investigated in the present study range from 11 to 911  $\mu\text{g}$   $\text{g}^{-1}$  fresh weight (Table 3). This compares with the 1.9–30  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  detected by Hertog *et al.* (1992a) in leaves of *L. sativa* var. Capitula, purchased in The Netherlands, and the 1–54  $\mu\text{g}$  of quercetin  $\text{g}^{-1}$  detected in 13 varieties of U.S.A.-grown head and leaf lettuce by Bilyk and Sapers (1985).

**Analysis of Endogenous Flavonoids in Celery.** In keeping with the report of Hertog *et al.* (1992b), preliminary studies indicated that acid-hydrolyzed extracts of celery contained significant quantities of api-

**Table 4. Luteolin and Apigenin Content of Commercial Celery (*Apium graveolens*) Purchased at Different Dates between August 1994 and April 1995**

material	purchase date	( $\mu\text{g}$ $\text{g}^{-1}$ fresh weight)	
		luteolin	apigenin
white celery stalks			
var. Greensleeves	3 Aug 1994 <sup>a</sup>	40	90
var. Ista	12 Jan 1995	36	104
var. Ista	23 Jan 1995	nd <sup>b</sup>	nd
var. Celebrity	6 May 1995	nd	49
green celery hearts			
var. Victoria	30 Jun 1995	35	191
white celery hearts			
var. Celebrity	30 Jun 1995	6.6	17

<sup>a</sup> No internal standard used with Aug 1994 sample. <sup>b</sup> nd, not detected; limit of detection ca. 1  $\mu\text{g}$   $\text{g}^{-1}$  fw.

genin and luteolin and that quercetin and kaempferol were not present in detectable amounts. Quantitative estimates of the luteolin and apigenin content of celery purchased in a Glasgow supermarket between August 1994 and June 1995 are presented in Table 4. In white celery stalks purchased in August 1994 and May 1995, and one of two samples from January 1995, the amounts detected were in keeping with the 22  $\mu\text{g}$  of luteolin and 108  $\mu\text{g}$  of apigenin  $\text{g}^{-1}$  fresh weight reported for celery by Hertog *et al.* (1992b). However, in the present study significant sample-to-sample variation was observed. For instance, the white celery stalks var. Ista purchased on January 12, 1995, contained 36  $\mu\text{g}$  of luteolin and 104  $\mu\text{g}$  of apigenin  $\text{g}^{-1}$  while neither flavone was detected in the same material purchased 11 days later. Green celery hearts var. Victoria contained 35  $\mu\text{g}$  of luteolin and 191  $\mu\text{g}$  of apigenin  $\text{g}^{-1}$  while white celery hearts of var. Celebrity acquired at the same time contained only 6.6  $\mu\text{g}$  of luteolin and 17  $\mu\text{g}$  of apigenin  $\text{g}^{-1}$ .

**Effect of Cooking on the Quercetin Content of Tomatoes and Onions.** Data on the effects of cooking on the conjugated quercetin content of tomatoes and onions are presented in Table 5. With both tissues, microwave cooking resulted in a fall in quercetin content and boiling produced an even bigger reduction. The losses that occurred during frying were less substantial. It should be noted that the sunflower oil used for frying the tomato and onion samples did not contain free or conjugated flavonoids either before or after frying.

## DISCUSSION

The data presented are broadly in line with those published by Hertog *et al.* (1992a) from a Dutch study that included similar tissues. Both investigations have shown wide variations in the flavonoid content of individual samples of common vegetables, but in the present report it has been possible to identify some systematic, predictable variations.

**Table 5. Effect of Frying, Boiling, and Microwave Cooking on the Conjugated Quercetin Content of Scottish Tomatoes var. Spectra and White Onions<sup>a</sup>**

plant material	cooking	quercetin content	
		$\mu\text{g g}^{-1}$ fw $\pm$ SE	% of uncooked
tomatoes	none	7.1 $\pm$ 0.4	100
	fried	4.6 $\pm$ 0.8	65
	boiled	1.3 $\pm$ 0.1	18
	microwaved	2.5 $\pm$ 0.1	35
onions	none	342 $\pm$ 12	100
	fried	269 $\pm$ 22	79
	boiled	87 $\pm$ 7.2	25
	microwaved	124 $\pm$ 8.5	36

<sup>a</sup> Data expressed as  $\mu\text{g g}^{-1}$  fresh weight  $\pm$  SE ( $n = 3$ ) and as a % of the fresh, uncooked material.

The data obtained with tomatoes indicate large varietal differences in the levels of conjugated quercetin, the highest concentrations being found in cherry tomatoes at all sampling points (Table 1). Dutch beef tomatoes, and similarly Spanish- and Scottish-grown tomatoes, which are sold loose and in large quantities in U.K. supermarkets and fruiterers, contained relatively low concentrations of flavonoids compared to Spanish and English cherry tomatoes, which are more expensive and are not consumed so extensively. There were variations in the levels of quercetin in cherry tomatoes purchased at different times, with the Spanish fruit var. Paloma generally containing more than the English-grown equivalent, var. Favorita. Whether this is a reflection of changing seasonal growing conditions or other factors remains to be determined. There was less variation in the flavonoid content of onions which contained relatively high levels of conjugated quercetin (Table 2). Cooking both tomatoes and onions resulted in a lowered quercetin content although less so following frying than boiling or microwave cooking (Table 5). This could be due to flavonoid breakdown during cooking and/or conjugated quercetin being extracted from the tomato and onion tissues by hot water more effectively than with hot sunflower oil.

Varietal differences in the conjugated quercetin content of lettuce cultivars was even greater than that observed with tomatoes (Table 3). "Round" lettuce which is cheap and used widely in the U.K. contained about eight times less quercetin than "Lollo Bionda" and more than 50 times less quercetin than the red-leaved "Lollo Rosso". The two home-grown cultivars that were investigated, "Green Salad Bowl" and "Marvel of Four Seasons" both had conjugated quercetin levels well in excess of the "Round" lettuce. The high quercetin content of the leaves of the anthocyanin-rich "Lollo Rosso" compared to its green equivalent "Lollo Bionda" may well be a consequence of the proximity of the flavonol and anthocyanin biosynthesis pathways (see Holton and Cornish, 1995) and warrants further study.

Different varieties of celery contained very variable levels of conjugated luteolin and apigenin. Although more detailed study is clearly required, the variations in apigenin and luteolin content of celery could be due to the interaction of a number of factors, including varietal differences, the light regimes under which the plants were grown, as UV-B irradiation is known to induce the accumulation of flavonoids (Li *et al.*, 1993; Lois, 1994), and the conditions under which the plant materials were stored during transport from the grower to the supermarket.

The results presented in this paper are of potential importance in view of recent epidemiological studies indicating that flavonoid intake is associated with a reduced risk of cancer, coronary heart disease, and stroke. It is estimated that at least 20% of coronary heart disease is attributable to diet and dietary factors are considered responsible for 40–60% of cancer incidence and 35% of cancer deaths (National Research Council, 1989). Fruits and vegetables have consistently been found to be protective against coronary heart disease (Hertog *et al.*, 1993a, 1995) and against a variety of cancers (National Research Council, 1989; WHO, 1990). The quantitative roles of antioxidants are not known precisely in relation to their health benefits, nor are the specific contributions of carotenoids, tocopherols, and ascorbic acid. However, recent evidence obtained with an *in vitro* oxidation model for heart disease has demonstrated that several plant flavonols, such as quercetin, myricetin, and rutin are more powerful antioxidants than the traditional vitamins (Vinson *et al.*, 1995). The health influences of flavonoids have yet to be fully established although they have been shown to function in a way similar to antioxidant vitamins and to protect against lipoprotein oxidation *in vitro* (Negre-Salvayre and Salvayre, 1992) and to have anti-platelet anti-thrombotic actions (Gryglewski *et al.*, 1987; Cooke and Samman, 1996). There are, therefore, grounds for encouraging the use of foods rich in flavonoids. It is evident with tomatoes and lettuce, and in all probability with other species, that there are very large varietal differences in flavonoid content (Tables 1 and 3). Identification and incorporation of flavonoid rich foods into the diet is clearly one means whereby the intake of flavonoids derived from fruits and vegetables could be increased markedly.

The relative contribution of vegetables to total flavonoid intake will depend to a large degree on the consumption of other rich dietary sources of flavonoids, such as tea and wines (see Hertog *et al.*, 1993b). Tea is considered one of the main dietary sources of flavonoids for adults in the U.K., but its limited use by younger people is declining in favor of carbonated drinks and coffee, which are relatively low in flavonoids. Red wines are a major source of flavonoids in countries such as Italy and France where there is relatively little consumption of tea (Hertog *et al.*, 1995). In contrast, red wine is consumed by only a minority of the U.K. population. It is likely that the processes leading to coronary heart disease and cancers are initiated many years before the diseases manifest themselves. If flavonoid intake is important in children, who do not consume tea or wine in any quantity, then the varieties of fruits and vegetables they consume could be critical for future health.

If it is accepted that higher intakes of flavonoids from foods are associated with long-term health benefits, then the data presented in this paper offer possible avenues for horticultural approaches toward health promotion, by identifying and selecting varieties rich in flavonoids, by optimizing growth and storage conditions and through advice on cooking. It should be noted that accurate measurement of flavonoids in foods is relatively cheap and not especially time consuming, and thereby offers a novel method for product quality assurances.

#### ACKNOWLEDGMENT

M.S.M. was supported by funds provided by the University of Glasgow Faculty of Medicine. C.B. was in receipt of a Flora project summer studentship. We

thank Mrs A.V. Sutcliffe for supplying fresh samples of home-grown lettuce and Dr. C.T. Wheeler, Department of Biochemistry and Molecular Biology, University of Glasgow for the use of the Modulyo freeze-drier which was purchased with a grant from the Royal Society. We would also like to acknowledge the invaluable assistance we received from Dr. G. Bailey (Research and Development, Safeway Stores plc, Hayes, U.K.) which enabled us to identify the individual varieties of tomato, lettuce and celery that were analyzed in this study.

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Received for review May 22, 1996. Revised manuscript received December 2, 1996. Accepted December 12, 1996.®

JF960339Y

® Abstract published in *Advance ACS Abstracts*, February 1, 1997.