Composition and Content of Flavonol Glycosides in Green Beans and Their Fate during Processing

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Four varieties of green bean (*Phaseolus vulgaris*) were analyzed for flavonol composition and content. Two flavonol conjugates, not previously reported in green bean, were found in three of the varieties. These are quercetin 3-*O*-(2^G-*β*-D-xylopyranosylrutinoside) (**1**), (1.0−2.0 *μ*g/g of fresh weight) and the corresponding kaempferol analogue (**2**) (0.3−0.7 *μ*g/g). The major flavonol component in all of the varieties was quercetin 3-*O*-glucuronopyranoside (**3**) (3.5−15.1 *μ*g/g) with lesser amounts of quercetin 3-*O*-rutinoside (**4**) (0.2−4.3 *μ*g/g) and kaempferol 3-*O*-glucuronopyranoside (**5**) (0.5−1.3 *μ*g/g). Kaempferol rutinoside (**6**) was detected in only one variety (0.8 *μ*g/g). Commercial processing, such as canning, did not result in chemical breakdown of the conjugates, although between 8.8 and 24.4%, depending on the conjugate, was leached into the cooking water.

Keywords: *Phaseolus; green bean; HPLC; flavonol; glycoside*

INTRODUCTION

A diet containing a higher proportion of fruits and vegetables has for some time been advocated in the battle to reduce the incidence of heart disease in the Western world (Block, 1992). The protective effect, which this type of diet confers, is believed to be due, in part, to the antioxidant activity of the flavonoids, a class of compounds found in most fruits and vegetables (Hertog et al., 1993). The antioxidant activity of one of the subclasses of the flavonoids, the flavonols, has been reported to be greater than that of either vitamins C or E (Rice-Evans et al., 1995).

There are five foods in the U.K. diet, namely, tea, onion, broccoli, apple, and green bean, which supply the majority of the flavonols in our diet [by comparison with data obtained in The Netherlands (Hertog et al., 1993)], and to further understand the mode of action of these flavonols, as phytoprotectants, both their composition and content in these foods have been the subject of much research (Hertog et al., 1993; Price and Rhodes, 1997; Price et al., 1997, 1998; Crozier et al., 1997).

The flavonols in these foods are based on three aglycons, myricetin, quercetin, and kaempferol, although they are present only in the edible portions of the food plants in the form of a multiplicity of conjugates. Recent work demonstrates the importance of the chemical nature of these conjugates to their bioactivity and bioavailability. These findings suggest the degree of hydroxylation is important in deciding the antioxidant activity (Plumb et al., 1997) as is the ability to induce phase II enzymes such as quinone reductase (Uda et al., 1997), whereas the type and degree of glycosylation may be important in determining the ability of these compounds to cross the intestinal wall (Gee et al., 1998).

This work reports on both the composition and content of the flavonol conjugates found in four commercial varieties of green bean grown in the United Kingdom. Two of the six flavonol conjugates measured were found to be novel to both green beans and the human food chain. These novel compounds are in three of the four varieties tested. The effect of a commercial canning process on the fate of these flavonols is also reported.

MATERIALS AND METHODS

Green beans (*Phaseolus vulgaris*), varieties Labrador, Matador, Lasso, and Montano, were grown on a commercial scale in Norfolk and collected at the time of normal commercial harvest, which was between August 19 and 28, 1998. All solvents were of AnalaR or HPLC grade when appropriate. MN polyamide SC6 was purchased from Macherey-Nagel GmbH & Co. Canned beans were produced in a local food processing factory under normal operating conditions, which involved washing, slicing, and cooking in the can with salted water. The sample of uncooked beans used as a control was from the same batch that was used in the canning process.

Quantitative Extraction. Raw beans (500 g) for each variety and beans drained from three cans of cooked beans (431.9 g) together with the cooking water (473.7 g) were each freeze-dried for subsequent quantitative extraction and analysis. Each sample was ground to a fine powder in a domestic food processor and extracted as follows: Duplicate samples of the dry powder (2 g) were homogenized three times in 70% methanol (50 mL) at 1200 rpm for 1 min (Pro400 homogenizer), and the homogenate was filtered under reduced pressure through filter paper (Whatman No. 1). The combined fractions were evaporated in vacuo at 40 °C to ∼10 mL and made up to 20 mL with water. Aliquots (5 mL) were added to a polyamide (1 g) column preconditioned with methanol (20 mL) followed by water (60 mL). The column was washed with water (20 mL) and further eluted with methanol (40 mL) to elute the neutral flavonols and with methanol/20 M ammonia (99.5:0.5, 40 mL) to elute the acidic flavonols. Each extract was

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evaporated to dryness under reduced pressure at 40 °C and redissolved in a methanolic solution containing daidzein as an internal standard (8 μg/mL, 2 mL) and filtered prior to duplicate HPLC analyses (20 μL).

Isolation of Flavonol Conjugates. Fresh beans (var. Labrador, 1 kg) were freeze-dried and powdered. Ten portions (100 g) were each homogenized three times in 70% methanol (650 mL and cooled in ice) at 1200 rpm for 5 min (Pro400 homogenizer), and the homogenate was filtered under reduced pressure through filter paper (Whatman No. 541). The combined fractions were partially evaporated in vacuo at 40 °C (1 L) to remove the methanol, and the resultant aqueous phase was defatted by serial extraction with three portions of hexane (750 mL). The aqueous layer was fractionated in two portions (500 mL) on a polyamide column (100 g) that had been preconditioned with methanol (1 L) followed by water (2 L). The column was washed with water (1 L) and further eluted, under a pressure of 10 psi nitrogen, with methanol (1.2 L) to elute the neutral flavonols and with methanol/ammonia (99.5:0.5, 1 L) to elute the acidic flavonols. Each extract was evaporated to dryness under reduced pressure at 40 °C to yield 2.17 and 1.59 g, respectively, for the neutral and acid fractions.

High-Pressure Liquid Chromatography (HPLC). *Preparative HPLC.* A Prodigy 5u ODS3 reversed phase silica (250 mm by 21.2 mm i.d., Phenomenex Ltd., Macclesfield, U.K.) column was used with an isocratic solvent [8:2 acetonitrile/0.1% trifluoroacetic acid (TFA)] at a flow rate of 5 mL/min. The column effluent was monitored at 270 and 370 nm, and fractions were collected using a Gilson fraction collector.

Analytical HPLC. A Hewlett-Packard 1050 system comprising autosampler and quaternary pump coupled to a diode array detector and controlled by Chemstation software was used with a solvent gradient of A [water/tetrahydrofuran (THF)/TFA 98:2:0.1] and B (acetonitrile) used in the proportion of 17% B for 2 min increasing to 25% B after 5 min, to 35% B after a further 8 min, and to 50% B after 5 min. A column cleanup stage was used by increasing B to 90% after a further 5 min and finally reequilibration for 20 min at 17% B. The column used was packed with Prodigy 5u ODS3 reversed phase silica (250 mm by 4.6 mm i.d., Phenomenex Ltd.), and the effluent (1 mL/min) was monitored by a diode array detector.

Mass Spectrometry. Atmospheric pressure chemical ionization (APCI) spectra were obtained by continuous flow injection on a Platform benchtop mass spectrometer (Micromass, Manchester, U.K.) operated in the positive ionization mode. The mobile phase was 60:40 water/acetonitrile at a flow rate of 200 μL/min. Typical tuning parameters were as follows: corona, 3.00 kV; high voltage lens, 0.10 kV; cone, 10 V; source temperature, 130 °C; and APCI probe temperature, 550 °C.

NMR Spectroscopy. NMR spectra were obtained using a JEOL GX400 spectrometer operating at 400 MHz for ^{1}H and at 100 MHz for ^{13}C. Samples were dissolved in methanol-d_4, and all spectra were run at 27 °C. Chemical shifts are given relative to TMS but were measured using the solvent methyl signal as secondary reference (δ values: ^{1}H 3.30 ppm; ^{13}C 49.0 ppm). Phase sensitive 2D COSY and C/H correlation spectra were obtained for **1** and **4**. Acquisition parameters were as follows: COSY, 2048 (t_2) × 256 (t_1) data points and spectral width 3000 Hz; C/H correlation, 4096 (t_2) × 64 (t_1) data points and spectral widths 8000 Hz (^{13}C) and 1000 Hz (^{1}H). In the C/H correlation experiment the ^{1}H parameters were set to cover the carbohydrate region only, assignments outside this region being made from literature values for related compounds.

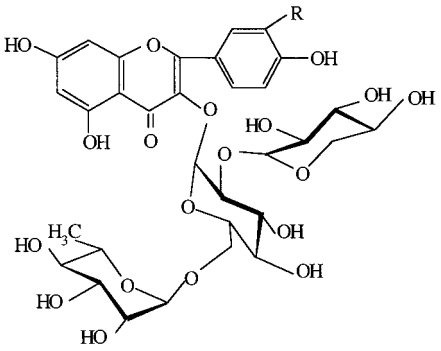
RESULTS

Identification of Flavonols. Four major components were observed in a chromatogram of the methanolic extract (from the polyamide column) of Labrador green bean, and these four components were isolated using preparative HPLC. Two of flavonols, when compared with the chromatographic behavior and UV spectral properties of standard compounds, were identified as the rutinosides of quercetin (**4**) and kaempferol (**6**) (retention times = 9.62 and 11.08 min, respectively). The identity of the former, **4**, was confirmed from both mass and NMR (Table 2) spectral measurements and by comparison with reported NMR parameters for rutin (Vasange et al., 1997).

Table 1. Mass Spectral Data for the Isolated Flavonol Conjugates

compound	*m/z*	% abundance	assignment[a]
quercetin triglycoside	743	16.5	MH^+
	611	10.2	$MH\text{-}p^+$
	597	2.4	$MH\text{-}d^+$
	465	5.5	$MH\text{-}p\text{-}d^+$
	303	100	$MH\text{-}p\text{-}d\text{-}h^+$
kaempferol triglycoside	727	18.1	MH^+
	595	7.1	$MH\text{-}p^+$
	581	1.6	$MH\text{-}d^+$
	449	4.7	$MH\text{-}p\text{-}d^+$
	287	100	$MH\text{-}p\text{-}d\text{-}h^+$
quercetin uronide	479	10.2	MH^+
	303	100	$MH\text{-}u^+$
quercetin rutinoside	611	49.6	MH^+
	465	11.8	$MH\text{-}d^+$
	303	100	$MH\text{-}d\text{-}h^+$
kaempferol uronide	463	11.0	MH^+
	287	100	$MH\text{-}u^+$

[a] p, pentose; d, deoxyhexose; h, hexose; u, uronic acid.



R =	Compound
-H	Kaempferol 3-O-xylosyl-(1->2)-rhamnosyl-(1->6)-glucoside. (**2**)
-OH	Quercetin 3-O-xylosyl-(1->2)-rhamnosyl-(1->6)-glucoside. (**1**)

Figure 1. Structures of flavonol conjugates.

The two more polar components (**1**, **2**; structures are given in Figure 1) isolated (retention times = 7.27 and 8.91 min, respectively) were tentatively identified from their UV spectra as quercetin (260, 270sh, 355 nm) and kaempferol (265, 355 nm) conjugates, respectively. Mass spectral data (Table 1) showed the conjugation in each case to comprise three sugars, a hexose, deoxyhexose, and a pentose. The sequential losses in the fragmentation pattern suggested both the pentose and deoyhexose to be terminal sugars to the hexose. The ^{1}H and ^{13}C NMR data (Table 2) confirmed that the aglycons were quercetin (**1**) and kaempferol (**2**). In particular, the C-2 and C-3 chemical shifts were very similar to those reported for quercetin and kaempferol glycosides from tea (Finger et al., 1991), with an oligosaccharide chain linked via the 3-position of the aglycon. A characteristic feature of substitution at this position is that the chemical shift displacement of C-2 (compared with the unsubstituted aglycon) is greater

Table 2. ^{1}H and ^{13}C NMR Chemical Shifts of the Flavonol Conjugates

		1 quercetin		**2** kaempferol		**3** quercetin		**4** quercetin		**5** kaempferol	
		δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H	δ^{13}C	δ^1H
	2	158.67		158.75		159.01		159.31		159.24	
	3	134.96		134.84		135.41		135.63		135.40	
	4	179.43		179.46		179.19		179.37		179.29	
	5	163.06		163.06		162.94		162.92		163.02	
	6	99.82	6.20	99.87	6.19	99.91	6.18	99.94	6.20	99.97	6.20
	7	165.70		165.71		165.96		165.96		166.03	
	8	94.75	6.39	94.83	6.38	94.76	6.37	94.86	6.39	94.83	6.39
	9	158.43		158.47		158.38		158.46		158.49	
	10	105.77		105.79		105.61		105.62		105.69	
	1′	123.33		122.94		122.83		123.12		122.50	
	2′	117.55	7.64	132.36	8.05	117.21	7.63	117.70	7.66	132.30	8.05
	3′	145.92		116.18	6.89	145.90		145.80		116.10	6.87
	4′	149.64		161.37		149.86		149.76		161.61	
	5′	116.09	6.88	116.18	6.89	116.00	6.84	116.05	6.87	116.10	6.87
	6′	123.54	7.62	132.36	8.05	123.52	7.63	123.56	7.62	132.30	8.05
sugars β-Glc (**1**, **2**, **4**) or β-GlcA (**3**, **5**)											
	1	100.90	5.41	100.82	5.40	104.32	5.31	104.74	5.10	104.39	5.30
	2	81.95	3.71	82.0	nd	75.37	3.53	75.72	3.45	75.41	3.50
	3	78.21	3.59	78.22	nd	77.58[a]	3.47	78.17	3.40	77.53[a]	3.46
	4	71.40	3.28	71.44	nd	72.82	3.59	71.37	3.26	72.83	3.57
	5	77.07	3.32	77.08	nd	77.02[a]	3.75	77.19	3.30	77.05[a]	3.73
	6	68.11	3.39, 3.81	68.13	nd[b]	172.14		68.55	3.38, 3.79	171.92	
α-Rha	1	102.14	4.51	102.14	4.49			102.40	4.51		
	2	72.10	3.61	72.07	nd			72.07	3.61		
	3	72.25	3.50	72.30	nd			72.24	3.51		
	4	73.91	3.25	73.86	nd			73.92	3.26		
	5	69.70	3.43	69.70	nd			69.69	3.44		
	6	17.84	1.10	17.85	1.08			17.87	1.10		
β-Xyl	1	105.12	4.78	105.18	4.77						
	2	74.66	3.40	74.70	nd						
	3	76.84	3.40	76.85	nd						
	4	70.98	3.51	71.01	nd						
	5	66.50	3.25, 3.98	66.55	nd						

[a] Assignments may need to be exchanged. [b] nd, not determined.

than that of C-3. The ^{13}C chemical shifts of **1** and **2** in the carbohydrate region were essentially identical, showing that the two compounds had the same sugar compositions and linkages. In the ^{1}H spectra anomeric signals were observed for three sugar units at δ 5.41 (d, $J_{12} = 7.4$ Hz), 4.51 (d, $J_{12} = 1.2$ Hz), and 4.78 ($J_{12} = 6.6$ Hz: a four-line multiplet arose from second-order coupling effect with δ H-2 = δ H-3). The ^{1}H and ^{13}C chemical shifts of the three sugar units in **1** were determined by a combination of COSY and C/H correlation experiments. The sugar ring with δ H-1 at 4.51 had the same NMR parameters as the terminal α-Rha residue in rutin, **4**, whereas the ring with δ H-1 at 4.78 had a coupling pattern and chemical shifts typical of a terminal β-Xyl residue (Bock and Thogersen, 1982). The coupling pattern and chemical shifts of the third sugar ring (δ H-1 at 5.41) showed that it was a 6-linked β-Glc residue, but there were significant differences between the H-1/C-1 and H-2/C-2 chemical shifts compared with **4**. The 6 ppm downfield shift of C-2 in **1** with respect to C-2 in **4** suggests that the β-Glc ring in **1** is linked via O2 to the terminal β-Xyl as well as being linked to the aglycon and (via O6) to the terminal α-Rha, as in rutin. Thus, **1** is quercetin 3-*O*-(2^G-β-D-xylopyranosyl-rutinoside) and **2** is the kaempferol analogue. Isolation of **1** from different sources (Nishida et al., 1990; Webby, 1991; Lee et al., 1995) has been reported previously. A detailed NMR analysis (Lee et al., 1995) established the sugar linkages unambiguously and is in good agreement with the results in Table 2.

The major components isolated from the methanol/ammonia eluate of the polyamide column (**3**, **5**) were also isolated and tentatively identified from their UV spectra as quercetin (255, 265sh, 355 nm) and kaempferol (265, 355 nm) conjugates, respectively. Mass spectral data (Table 1) indicated that both conjugates contained a uronic acid. For the aglycons, the NMR spectra of **3** and **5** (Table 2) showed great similarity to those of **1** and **2**, respectively, indicating that the aglycons were indeed 3-*O*-substituted quercetin (**3**) and 3-*O*-substituted kaempferol (**5**). The spectra of the two compounds were almost identical in the carbohydrate region and corresponded to a single sugar unit, with values typical of a β-GlcA residue (δ H-1 5.3, d with $J_{12} = 7.5$ Hz; δ H-5 ~3.74, d with $J_{45} = 9.8$ Hz; δ C-6 ~172). Thus, **3** is quercetin 3-*O*-β-D-glucuronopyranoside and **5** is the analogous compound of kaempferol.

Effect of Variety on Flavonol Composition and Content. The levels of the individual flavonol conjugates together with the total levels of both quercetin and kaempferol conjugates are shown in Table 3 and Figure 2 for the four varieties Labrador, Matador, Lasso, and Montano. The quercetin conjugates dominate in all of the varieties, representing between 84.3 and 92.3% of the total flavonols, with kaempferol accounting for the remaining flavonol present; the main conjugate in all cases was the quercetin 3-*O*-glucuronopyranoside. There was a 4-fold difference in total flavonol content, which ranged from 5.90 μg/g for Labrador, to 10.92 μg/g for Montano, to 12.09 μg/g for Matador, and to 21.49 μg/g for the variety Lasso. The flavonol composition profiles were similar in Labrador, Matador, and Montano. However, Lasso was quite different in that the triglycosides of both quercetin and kaempferol were

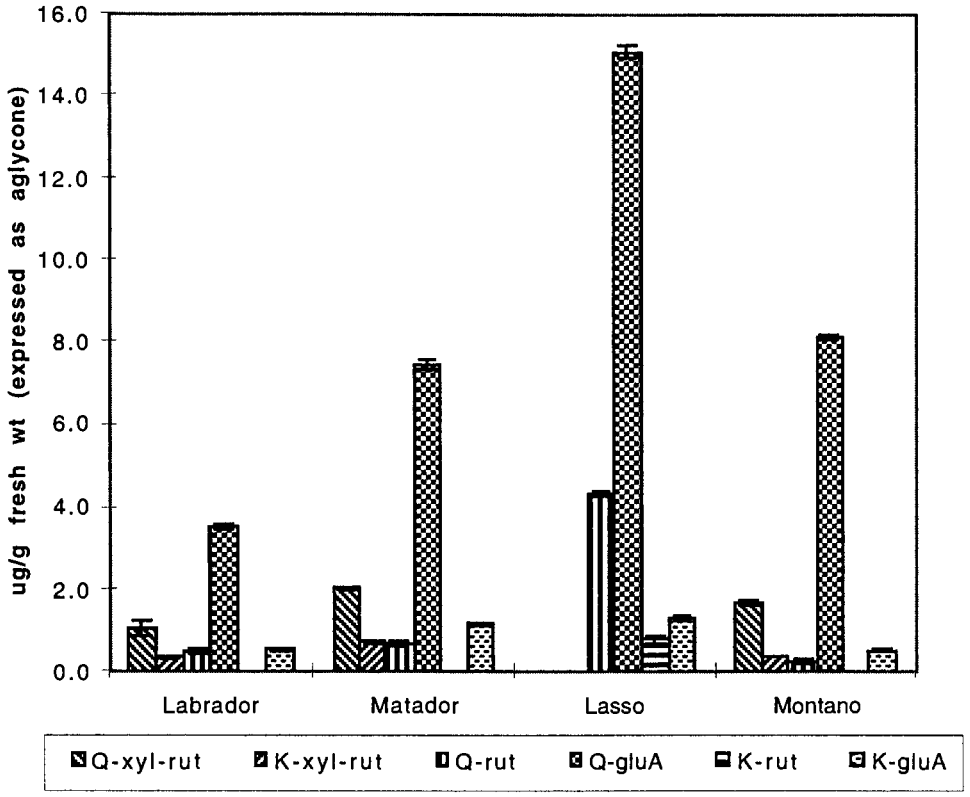


Figure 2. Levels of flavonol conjugates in four varieties of green bean.

Table 3. Flavonol Content (Micrograms per Gram of Fresh Weight as Aglycon) of Four Green Bean Varieties

compd no.	compd[a]	Labrador mean	Labrador SD	Matador mean	Matador SD	Lasso mean	Lasso SD	Montano mean	Montano SD
1	Q-xyl-rut	1.0	0.2	2.0	0.0	0.0	0.0	1.7	0.1
2	K-xyl-rut	0.3	0.0	0.7	0.0	0.0	0.0	0.3	0.0
4	Q-rut	0.5	0.0	0.7	0.0	4.3	0.0	0.2	0.0
3	Q-gluA	3.5	0.1	7.5	0.1	15.1	0.2	8.2	0.1
6	K-rut	0.0	0.0	0.0	0.0	0.8	0.1	0.0	0.0
5	K-gluA	0.5	0.0	1.2	0.0	1.3	0.0	0.5	0.1
	total flav	5.9	0.2	12.1	0.1	21.5	0.1	10.9	0.1
	total K	0.9	0.0	1.9	0.0	2.1	0.0	0.8	0.0
	total Q	5.0	0.3	10.2	0.1	19.4	0.2	10.1	0.1
	% Q	85.2	1.0	84.3	0.1	90.3	0.2	92.3	0.4

[a] Q, quercetin; K, kaempferol.

Table 4. Effect of Cooking on the Levels of Flavonol (Micrograms per Gram of Fresh Weight as Aglycon) in Green Bean Var. Labrador

compd no.	compd[a]	raw mean	raw SD	canned mean	canned SD	% loss
1	Q-xyl-rut	2.0	0.1	1.6	0.1	18.1
2	K-xyl-rut	0.5	0.0	0.4	0.0	20.4
4	Q-rut	0.6	0.0	0.5	0.0	18.4
3	Q-gluA	5.5	0.1	4.1	0.1	24.4
6	K-rut	0.0	0.0	0.0	0.0	0.0
5	K-gluA	0.5	0.0	0.5	0.0	8.8
	total flav	9.1	0.3	7.2	0.1	21.5
	total K	1.1	0.1	0.9	0.1	14.8
	total Q	8.1	0.2	6.3	0.1	22.4
	% Q	88.5	0.6	87.5	1.2	

[a] Q, quercetin; K, kaempferol.

absent. This variety, however, had higher levels of the rutinosides of both quercetin and kaempferol than the other varieties.

Effect of Processing on Flavonols. The levels and compositions of the flavonol conjugates in the raw beans (the variety used was Labrador), canned beans, and cooking water are shown in Table 4. The overall loss of flavonols from the beans to the canning water was 21.5%; the loss was 14.8% for the kaempferol conjugates and 22.4% for the quercetin conjugates. The transfer of individual conjugates varied from only 8.8% for the kaempferol 3-*O*-glucuronide to 24.4% for the quercetin 3-*O*-glucuronide. There was little change in either the composition or content of the flavonols when the data from the cooked beans were combined with that of the cooking and compared to the data from the raw beans, although the quercetin conjugates were more soluble than those of kaempferol and therefore were leached to a greater extent.

DISCUSSION

There is little information available in the literature regarding the nature of the flavonol conjugates in green beans apart from work carried out by Hempel and Bohm (1996), who identified four conjugates only, on the basis of HPLC and mass spectral data. These were the glucuronides and rutinosides of both quercetin and kaempferol in six varieties of green beans grown in Germany. In this paper, the isolation of the individual compounds and subsequent structure elucidation provide complete identification of the conjugates present in green beans and describe two compounds that have not previously been reported in green beans. These two conjugates, which are novel to a food plant, have been reported in several plants such as *Hosta ventricosa* (Budzianowski, 1990), *Scolymus hispanicus* (Rubio et al., 1995), and *Orixa japonica* (Nishida et al., 1990). In the latter example, the quercetin conjugate (**1**) was found to be a potent oviposition deterrent to the swallowtail butterfly, which may have implications for the

presence of this compound in three of the four varieties of bean pod studied here in terms of natural resistance to certain insects. The triglycoside moiety, β-D-xylopyranosylrutinoside, linked to the anthocyanidins cyanidin and delphinidin, is relatively common in fruits such as black raspberry, blackcurrent, and sour cherry (Shrikhande and Francis, 1973).

The variety Lasso did not have detectable levels of the two xylopyranosylrutinosides and was the only variety to contain kaempferol rutinoside together with a much higher level of the quercetin rutinoside.

The glucuronopyranosides of both quercetin and kaempferol have not been reported in the edible portions of other vegetables or bean seeds, although they are commonly found in fruits such as strawberry, blackberry, and raspberry (Macheix et al., 1990). In these fruits the presence of the xylosylglucuronopyranoside of both kaempferol and quercetin is also reported. However, no evidence for the presence of this conjugate was found for the green bean varieties studied here.

The quercetin and kaempferol contents of the green beans reported here of 5.0−19.4 and 0.8−2.1 μg/g of fresh weight, respectively, are somewhat lower than those reported by Hempel and Bohm (1996) of 9.5−90.8 and 2.7−7.1 μg/g of fresh weight, respectively, when the figures are expressed in terms of aglycon. However, because these workers also found large variations in the flavonol content within one variety over two growing seasons (1993 and 1995), the differences noted here between varieties would need to be recorded over different growing seasons before their significance could be assessed. The difference found in the flavonol content (but not the flavonol composition) in the two Labrador variety samples used in this study suggests that other agronomic conditions, such as soil type, may also be important.

In common with previous reports on the effect of cooking on both the composition and content of flavonol conjugates in onion (Price et al., 1997; Price and Rhodes, 1997) and broccoli (Price et al., 1998), the flavonol conjugates in green beans were stable during commercial cooking and canning, although as in the cases of both onion and broccoli there was a net loss of the conjugates from the cooked tissue to the cooking water (21.5%) and a larger proportion of the quercetin conjugates was lost (22.4%) as compared to the kaempferol conjugates (14.8%). This reduced loss of kaempferol was largely due to a much smaller loss of the kaempferol glucuronopyranoside (8.8%) when compared to that of the quercetin analogue (24.4%). The type of quercetin conjugate appeared to have a small effect on the degree of leaching, with the glucuronopyranoside (24.4%) being more available than the xylopyranosylrutinoside and rutinoside (18.1 and 18.4%, respectively).

Green beans have been identified as one of the five foods that provide the majority of the dietary flavonols in the United Kingdom, and this work shows green beans to contain not only flavonol glucuronopyranosides but also flavonol conjugates novel to the human food chain. The glucuronosides are typical detoxification products of the mammalian liver following normal gut absorption of many plant secondary metabolites, although the degree to which flavonol glucuronopyranosides are absorbed across the gut is not known. The nature of the conjugation in dietary flavonols appears to be a major factor in determining their bioavailability. For instance, the presence of a terminal glucose appears to be important in improving transport across the gut (Gee et al., 1998), as does the position of the glucose within the molecule (Hollman et al., 1995).

This work provides further information that is required to ultimately determine the biological significance of dietary flavonol conjugates in our diet.

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