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A comparison of the flavonol content and composition in dessert, cooking and cider-making apples; distribution within the fruit and effect of juicing

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Abstract

The fruit of all apple varieties tested possessed five quercetin glycosides, namely hyperin, isoquercitrin, reynoutrin, avicularin and quercitrin, as the major flavonol components. Total flavonol levels were in the range 26.4 to 73.9μg/g fresh wt (expressed as aglycone) with hyperin the dominant form in all varieties except Egremont and Jonagored, where quercitrin predominated, and the cider apples, where avicularin predominated. The proportion of flavonol in the peel ranged from 63.0 to 97.1% for the dessert and cooking apples and was not dependent on fruit size. Juice produced from the three varieties of cider apple contained 9.9 to 12.7% of the flavonols with the remainder retained in the pomace.

1. Introduction

An increased intake of fruit and vegetables in our diet has been advocated for a reduction in the incidence of heart disease in the Western World (Block, Patterson & Subar, 1992). The antioxidant activity of the flavonoids, a class of compounds found in most fruit and vegetables, is thought to be one of the contributing factors to this reduction of heart disease (Hertog, Feskins, Hollman, Katan & Kromhout, 1993; Keli, Hertog, Feskins & Kromhout, 1996; Knekt, Jarvinen, Reunanen & Maatela, 1996; Rimm, Katan, Ascherio, Shampher & Willett, 1996), whilst the antioxidant activity of one of the sub-classes of the flavonoids, the flavonols, has been reported to be greater than that of the vitamins C and E (Rice-Evans, Miller, Bolwell, Bramley & Pridham, 1995).

Five foods in the UK diet, namely tea, onion, broccoli, apple and green bean, supply the majority of the flavonols in our diet (Hertog et al., 1993) and the composition and content of the flavonols in these foods have been the subject of much research (Crozier, Lean, McDonald & Black, 1997; Hertog, Feskins et al., 1993; Hertog, Hollman & Venema, 1992; Price, Bacon & Rhodes, 1997; Price, Caususcelli, Colquohoun & Rhodes, 1998; Price, Colquohoun, Barnes & Rhodes, 1998; Price & Rhodes, 1997; Price, Rhodes & Barnes, 1998), although information on both, the level and composition of flavonols, in apple, is mainly concerned with apple peel rather than the whole fruit (Dick, Redden, DeMarco, Lidster & Grindley, 1987; Dong, Mita, Kootstra, Lister & Lancaster, 1995; Tueber, Wunscher & Hermann, 1978).

The flavonols in these foods are based on three compounds, myricetin, quercetin and kaempferol, although they are almost exclusively present in the edible portions of the food plants in the form of conjugates. The degree and location of hydroxylation within the aglycone is important in deciding their antioxidant activity (Jovanovic, Steenken, Hara & Simic, 1996; Plumb, Price, Rhodes & Williamson, 1997) and their ability to induce phase II enzymes such as quinone reductase (Uda, Price, Williamson & Rhodes, 1997), whilst the type and degree of glycosylation may be important in determining the ability of these compounds to cross the intestinal wall (Gee, Dupont, Rhodes & Johnson, 1998).

This work reports on both the composition and content of the flavonol conjugates in four varieties of eating apple, one variety of cooking and three varieties of cider making apple, and the distribution of these compounds in pomace and juice of the cider apples and between flesh and peel in the eating and cooking varieties.

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2. Materials and methods

2.1. Materials

2.1.1. Apples and apple products

Five varieties of apple (2 kg each), namely Granny Smith, Bramley, Cox's Orange, Jonagored and Egremont Russet, were grown in 1995 and supplied by Norfolk Fruit Growers, Wroxham, Norfolk, UK.

Three varieties of cider apple grown in 1997 and the resultant pomace and juice following commercial scale pressing, were supplied by HP Bulmers Ltd., The Cider Mills, Plough Lane Hereford HR4 0LE, UK.

2.1.2. Standards

Quercetin-3-*O*-rhamnoglucoside (rutin), quercetin-3-*O*-glucoside (isoquercitrin), quercetin-3-*O*-galactoside (hyperin), quercetin-3-*O*-xyloside (reynoutrin), quercetin-3-*O*-rhamnoside (quercitrin) and quercetin-3-arabinofuranoside (avicularin) were purchased from Apin Chemicals Ltd., Abingdon, UK.

Stock solutions were prepared in methanol and were stored at −10°C between analyses.

All solvents were either AnalaR or HPLC grade where applicable. Methanol was obtained from Fisons, Loughborough, UK; acetonitrile, trifluoroacetic acid (TFA), tetrahydrofuran (THF) and acetic acid from BDH, Poole, UK and the water was purified via a Millex Q-plus system, (Millipore Ltd, Watford, UK). MN polyamide SC6 was purchased from Macherey-Nagel GmbH and Co, Germany.

2.2. Methods

2.2.1. Eating and cooking apple samples

Five apples from each variety, freshly harvested and washed in cold water, were cut in quarters and cored. Peeled quarters and the peel were each weighed and immediately frozen by immersion in liquid nitrogen, freeze-dried, reduced to a powder in a domestic blender and stored at −10°C prior to extraction.

2.2.2. Cider apples and products

Whole apples, pomace and juice were frozen and stored at −40°C prior to extraction.

2.2.3. Solvent extraction of freeze-dried samples.

Each sample (2 g, in duplicate) was homogenised three times in a solvent mixture (50 ml) comprising methanol:water:acetic acid (70:30:5) at 1400 rpm for 1 min (Pro400 homogeniser, CT, USA) whilst cooled in ice. The homogenate was filtered under reduced pressure through filter paper (Whatman No. 1) and the combined fractions evaporated under reduced pressure at <45°C to approximately 10 ml and made up to 20 ml with water.

2.2.4. Solvent extraction of frozen apple and apple product samples

Whole frozen apple: four fruit of each sample (in duplicate) were weighed and immersed in methanol: acetic acid mixture (such that the water in the apples made a final solvent composition of 70:30:5 methanol:water:acetic acid), and homogenised in a blender for 2 min (Waring blender, Fisher Scientific, Loughborough, UK). Two further extractions of the residue (2×1l MeOH:water:acetic acid,70:30:5) for 2 min were bulked and evaporated under reduced pressure at <45°C to less than 200 ml and made upto 200 ml.

Pomace: The mass was allowed to thaw sufficiently to obtain a representative sample (300 g, in duplicate) which was then treated as for the frozen fruit except that the extract was made upto 220 ml.

Juice: The juice was allowed to thaw at 4°C, and representative aliquots (300 ml, in duplicate) were weighed prior to clean up.

2.2.5. Extract clean up

Two ml (in duplicate) of each aqueous extract was loaded onto a column containing polyamide (1 g) preconditioned with methanol (20 ml) and water (60 ml). The column was eluted sequentially with water (20 ml) and methanol (40 ml), the methanol fraction evaporated under reduced pressure at <45°C to dryness, redissolved in methanol (1 ml) and filtered (0.22 μm). Duplicate samples (10 μl) were used for HPLC analysis.

2.2.6. HPLC analysis

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: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 clean up stage was used by increasing B to 90% after a further 5 min and finally re-equilibration for 20 min at 17% B. The column used was packed with Prodigy 5 μm ODS3 reversed phase silica (250 mm by 4.6 mm id, Phenomenex Ltd., Macclesfield, UK) and the effluent (1 ml/min) was monitored by a diode array detector.

Compounds were quantified by their absorption at 370 nm. Retention times were rutin (9.4 min), hyperin (10.3 min), isoquercitrin (10.5 min), reynoutrin (11.3 min), avicularin (12.0 min) and quercitrin (12.3 min). Peak purity was assessed by comparison of UV spectra of both leading and trailing peak edge and with those of the standard compounds.

A linear response was found for each compound in the range 0–10 μg injected and only peak areas within this range were used. A mixture of the standards was analysed between each sample and used as an external

standard mixture. Mean response of the 6 standard compounds was a peak area of 2.6797 per ng injected (error less than ±1.33% for quadruplicate injections) in terms of quercetin except for quercitrin which was not pure by hplc.

3. Results

The total flavonol contents in the eight varieties of apple studied together with the average fruit size are given in Table 1. The levels ranged from 26.4 to 73.9 μg/g fresh weight and were not related to fruit size.

The compositions of the individual flavonol conjugates in each of the eight varieties of dessert, cooking and cider apple are given in Table 2. Six quercetin conjugates: rutin (Q-3-*O*-rutinose), hyperin (Q-3-*O*-galactose), isoquercitrin (Q-3-*O*-glucose), reynoutrin (Q-3-*O*-xylose), avicularin (Q-3-*O*-arabinofuranose) and quercitrin (Q-3-*O*-rhamnose), were measured in the apples, although rutin was only detected in the cider apples (Fig. 1). Hyperin was the dominant conjugate in Cox's Orange, Bramley and Granny Smith whereas quercitrin dominated in Jonagored and Egremont. Isoquercitrin was the minor conjugate in all varieties except Egremont where hyperin was the minor component. The distribution of total flavonol content between flesh and peel for the five varieties of dessert and cooking apples is shown in Table 3. In all of the varieties, the flavonols were concentrated in the peel although the proportion in the peel varied between a high of 97.1% in Granny Smith to a low of 63.0% in in Egremont.

The distributions of the individual conjugates beween the flesh and peel are shown in Table 4. The variety Egremont was unusual, with a significant total flavonol content (37%) in the flesh of the fruit, although this was largely due to 76% of the isoquercitrin residing in the flesh. In the other four varieties, quercitrin was always present in the flesh in a higher proportion when compared to that in the whole fruit.

The distribution of total flavonols between juice and pomace in the 3 varieties of apple used for juicing is given in Table 5. Although the apple fruit is converted into juice and pomace in an approximate proportion of 3 parts of juice to 1 part of pomace by weight, the juice contains only a relatively small proportion of the flavonols at levels of 3.2 to 5.0 μg/g, with the majority of the flavonol conjugates in the pomace at levels of 87.0 to 113 μg/g. There was a preferential transfer of isoquercitrin to the juice observed for Yarlington (Fig. 2) but no change observed in the flavonol conjugate profile for either the pomace or the juice in the other two cider varieties.

Table 1
Total flavonol content and average fruit weight (g) in eight varieties of apple (μg/g fresh weight expressed as aglycone)

Variety	Fruit size (g)	Content μg/g fresh wt (as quercetin)	SD
Eating apples:			
Egremont	86	26.4	1.6
Cox's orange	111	69.5	0.5
Granny Smith	121	40.5	2.2
Jonagored	175	73.9	0.6
Cooking apple:			
Bramley	235	61.3	1.5
Cider apples:			
Dabinett	78	32.5	0.3
Michelin	71	40.9	0.8
Yarlington	89	41.9	2.2

4. Discussion

The range of total flavonol content found in whole fruit for this study (26.4–73.9 μg/g) is difficult to compare with the quantitative results obtained by other workers since they are exclusively concerned with the levels of the flavonols in the skin of the apple fruit, where increases in flavonol content are known to be induced by changes in variables such as visible and UV light exposure (Lancaster, 1992). The general consensus concerning the distribution of flavonols in apple fruit

Table 2
Individual flavonol conjugate content for the eight varieties of apple (μg/g fresh weight expressed as aglycone)

Apple variety	Content of individual flavonol glycosides (μg/g fresh weight expressed as aglycone)											
	Q-rut	SD	Q-gal	SD	Q-glu	SD	Q-xyl	SD	Q-ara(f)	SD	Q-rha	SD
Egremont	nd		1.2	0.5	3.3	0.2	2.0	0.4	4.9	0.6	15.0	0.3
Cox's orange	nd		29.7	0.3	3.7	0.2	10.4	0.1	17.8	0.4	7.9	0.4
Granny Smith	nd		12.0	0.2	4.2	0.7	5.1	0.9	8.8	1.0	10.5	1.0
Jonagored	nd		18.7	0.4	4.3	0.2	9.7	0.1	18.9	0.2	22.4	0.6
Bramley	nd		21.1	0.3	7.4	0.3	8.4	0.2	15.0	0.2	9.4	0.2
Dabinett	0.4	0.0	9.5	0.1	3.1	0.0	4.3	0.0	10.4	0.1	4.8	0.2
Michelin	2.7	0.1	11.5	0.2	3.6	0.1	5.9	0.1	13.3	0.2	3.8	0.3
Yarlington	0.7	0.0	9.6	0.5	3.9	0.2	5.1	0.3	15.4	0.8	7.2	0.4

nb: rut = rutinoside; gal = galactoride; glu = glucoside; xyl = xyloside; ara = arabinoside; rha = rhamnoside; SD = standard deviation.

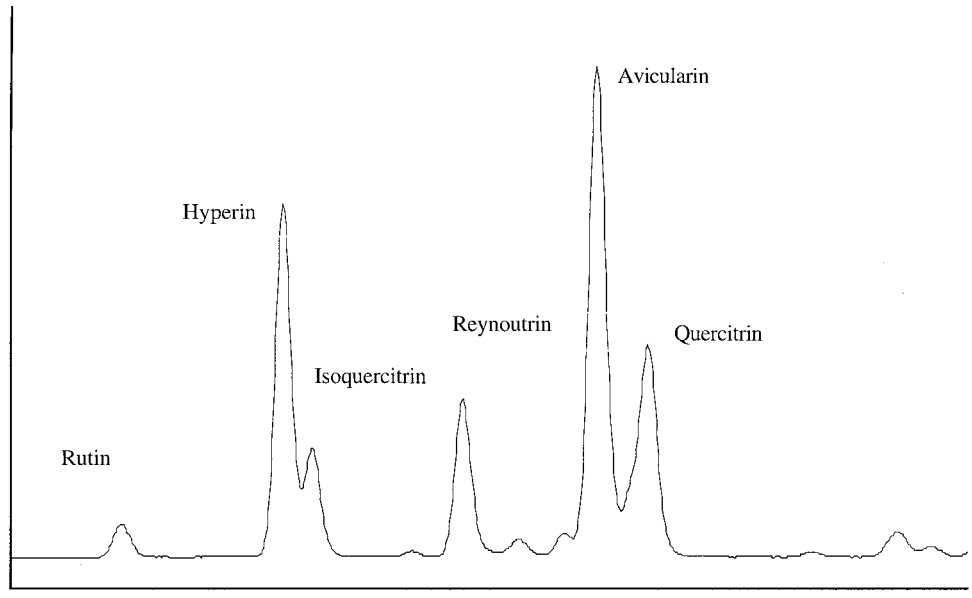

Fig. 1. Typical chromatogram of apple fruit extract.

Table 3
Total flavonol distribution between peel and flesh for five varieties of apple (μg/g fresh weight of whole fruit expressed as aglycone)

Variety	Fruit size (g)	wt% peel	wt% water	Flavonol in flesh	Content in peel	%wt flavonol in peel
Eating apples:						
Egremont	86	19.2	83.6	12.1	86.6	63.0
Cox's orange	111	15.8	81.0	3.9	419	95.3
Granny Smith	121	15.3	87.2	1.4	256	97.1
Jonagored	175	18.5	82.4	12.3	345	86.4
Cooking apple:						
Bramley	235	16.0	86.5	3.1	366	95.8

Table 4
Individual flavonol conjugate distribution between peel and flesh for five varieties of apple (μg/g fresh weight of whole fruit expressed as aglycone)

Variety	% (by wt) of flavonol conjugate in peel (μg/g whole fruit)				
	Q-gal	Q-glu	Q-xyl	Q-ara	Q-rha
Egremont	100.0	24.2	70.0	83.7	60.7
Cox's orange	98.3	91.7	96.2	96.1	83.5
Granny Smith	100.0	100.0	100.0	100.0	88.6
Jonagored	98.4	83.7	88.7	93.6	70.1
Bramley	98.1	95.9	95.2	97.3	89.4

nb: gal = galactoride; glu = glucoside; xyl = xyloside; ara = arabinoside; rha = rhamnoside.

is that they reside exclusively in the skin and that peeling of the fruit would reduce the flavonol content to very low levels (Lister, Lancaster, & Walker, 1996). This work shows that normal domestic peeling, of the fruit of the five varieties of dessert and cooking apple grown in the UK and studied here, resulted in losses ranging from 63.0 to 97.1% and that the individual flavonol conjugates are not necessarily distributed in the same proportions between flesh and peel of the fruit.

Table 5
Distribution of total flavonol content between pomace and juice for three varieties of cider apple (wt% in peel)

Variety	Flavonol content (μg/g fresh wt as aglycone)		
	Whole fruit	Pomace	Juice
Dabinett	34.3	112.9	4.3
Michelin	40.9	87.0	3.2
Yarlington	45.2	103.0	5.0

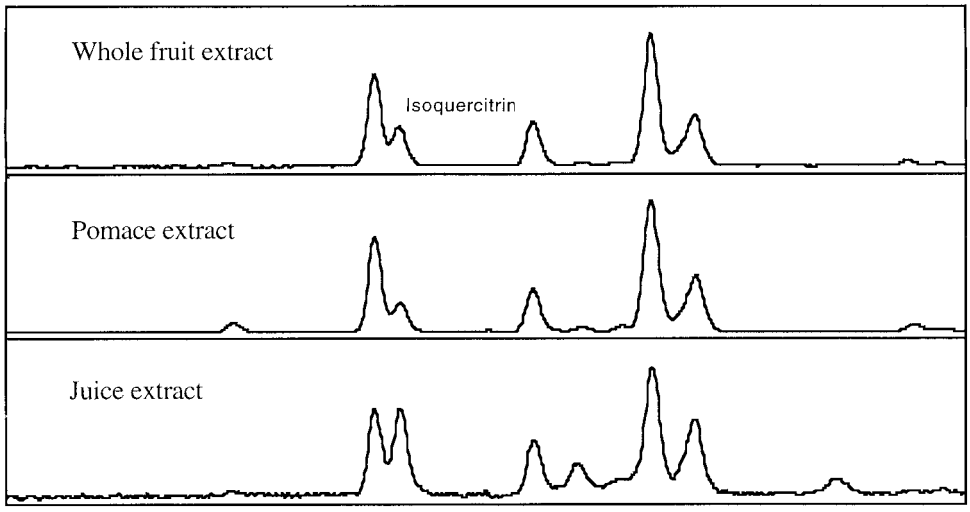

Fig. 2. Chromatograms of fruit, pomace and juice extracts (Yarlington variety).

Since apple is one of the major dietary sources of flavonols in the UK diet (Hertog et al., 1993), the consumption of a variety such as Egremont, even when peeled, would still result in a significant intake of the flavonol conjugates. However, in the case of juicing, the majority of the flavonols are concentrated four-fold in the pomace, which gives the potential of the pomace as a rich source of flavonols. The importance of the type of conjugation of the flavonols, in terms of their bioavailability during digestion, has recently been demonstrated (Gee et al., 1998; Hollman, deVires, van Leeuwen, Mengelers, & Katan, 1995), which suggests that quercetin conjugated to glucose, in contrast, for example to rhamnose, is preferentially absorbed via the small intestine, although further studies are required before the full mechanism of intestinal absorption of these natural antioxidants is understood. This could have implications for flavonol rich foods marketed for their beneficial properties.

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